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EVOLUTION OF THE BASIDIOMYCETES AND ITS RELATION TO THE TERMINOLOGY OF THE BASIDIUM

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(WITH 6 FIGURES)

Within the past fifteen years especially, but dating back to Patouillard's (1900) work on the classification of the Basidiomycetes, interest in the morphology of the basidium has increased greatly. This is to be expected when that fundamental structure, because of the increased importance placed upon microscopical characters, has come in for careful scrutiny. Since the basidium is an integral and a most important structure in relation to the nuclear cycle of the forms, it is obvious that its fundamental nature should make it a logical organ in which to search for characteristics to employ for the separation of this large class of fungi into the smallest possible groups, and at the same time to show their natural relationships.

It seems unfortunate, however, once the characteristics of the basidium have been fairly completely learned, that there must also be an increase in terminology beyond our actual needs and as a result mycologists are faced with the necessity of endeavoring to ascertain exactly what various writers mean when they apply the terms probasidium, hypobasidium, promycelium, metabasidium, epibasidium, and sterigmata in the various orders of the Basidio-

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mycetes and more especially in the lower groups. To the writer it seems that the greatest service is rendered, not by the creation of new names, but by the employment of the smallest number of terms that are compatible with a clear and consistent definition. For this reason, then, the older terminology seems amply to satisfy the needs of the investigator. Thus the term *probasidium* is fully adequate to describe the resistant bodies represented by the teleutospores of the Uredinales (or in exceptional cases the aeciospores), the chlamydospores of the Ustilaginales, or the more or less resistant bodies of the Auriculariales. The name *basidium* then would be applied to that structure which is in part the homologue of the ascus and which, following caryogamy and meiosis, bears the spores either directly or indirectly through the interpolation of the sterigmata. Recognizing that there are variations, it may be necessary to have further refinement of terms and to utilize such terms as *phragmobasidium* in connection with the type of basidium presented by the Uredinales, Ustilaginales, and Auriculariales, or *cruciate basidium* for the Tremellales, and *holobasidium* for the higher Basidiomycetes. If there is interpolated between the probasidium and the basidium a mycelial phase which may be more or less extensive, as is true in the genera *Helicogloea* or *Myxomycidium*, or even in some species in the Uredinales, it is sufficient to realize that the basidium is stalked and to speak of that structure as the *basidial stipe*. This stipe is not a new structure of fixed length for a given species, but rather varies in length according to conditions of the environment. Thus in *Gymnosporangium Nidus-avis*, if the spores are germinated in water, it will be found that the stipe of the basidium arising from those teleutospores that are near the surface of the water are very short, whereas the stipe arising from teleutospores more deeply immersed are proportionately more elongate. Similarly, when probasidia are deeply immersed in a gelatinous matrix, as in *Helicogloea*, the length of the stipe is proportional to the depth to which the probasidium is immersed. The writer is in agreement with Martin (**1938**) when he writes, "It seems clear that Boedijn's contention that the two parts of the basidium in *H. indicum* are separate organs can not be admitted." Finally, the term *sterigma*, which like the basidium has been refined almost beyond recognition, would

be applied to that structure which arises from the basidium and bears the basidiospore. Because of the homologies to be pointed out later in this paper, the restriction of the sterigmata to apply only to that portion of the spore-bearing filament which is very slender and through which the nucleus squeezes in its passage to the spore, not only leads to confusion but is illogical. The same arguments used against considering the basidial stipe as a distinct organ are applicable here,—namely its development and its length are dependent on environmental factors such as immersion in a surface film of water or immersion of the basidium in a gelatinous matrix. Thus in this respect the writer is in full support of Boedijn's statement (p. 194, **1937**) that "Now it is clear that the 4-celled part [of the basidium of the Tremellales] certainly is homologous with the 4-celled basidium of the *Auriculariaceae*; only the septation is different, being vertical instead of transverse. According to the theory of Neuhoff we are forced, however, to accept the hypobasidium as one-celled and the epibasidium as 4-celled for the *Auriculariaceae* and just the reverse for the *Tremellaceae*." A glance at a section of the hymenium of *Auricularia Auricula-Judae* or a germinating teleutospore would show immediately the futility of Neuhoff's (**1924**) concept of what a sterigmata should be. This, even without the homologies to be pointed out later, is sufficiently inconsistent to drive one back to the old-fashioned concept of the sterigmata.

To make clearer the application of these terms, and to lend supporting evidence, it seems necessary to survey the origin of and the evolutionary trend within the Basidiomycetes. Before commencing this survey, however, it should be strongly emphasized that today we are dealing not with organisms that are the result of recent modification, but those which are in reality the end products of a long line of evolution in which the ancestral forms have been lost. As a result, today we can deal only with those forms which have retained some, or possibly all, of their primitive characteristics. For this reason the writer wishes to emphasize that when it is stated that one genus or group arose from another, the implication is in reality that what is considered to be the primitive form as it is found today, behaves in much the same manner as its hypothetical and geologically more ancient

progenitor, and not necessarily that a stated present day species or genus has given rise to another. Because of the almost complete lack of fossil evidence, it is necessary to evaluate the evidence that is furnished by living forms, a fact that has not been sufficiently kept in mind and which has led to many and often conflicting ideas in regard to the phylogeny of the Basidiomycetes. In order to obtain not only a standard nomenclature for the basidium and related parts, but also to secure a stability in taxonomy, it is necessary to accumulate as much evidence as possible so that from it there may be sorted out the material which will serve to build the basic structure of the much-to-be-desired system of natural classification. It is the writer's hope that the ideas expressed in the following pages may contribute to some extent towards this end.

ORIGIN OF THE UREDINALES

Where should we look for the ancestral forms of the Basidiomycetes? Because of the striking analogy shown by diagrams of the life-history of certain members of the red algae and of the Uredinales, it has more than once been suggested that we look for ancestral types in the Rhodophyceae. Without going into too great detail, this source may be discarded for several reasons which may be listed briefly as follows: (1) those forms of red algae which show the greatest similarity of life-cycle to that of the Uredinales are all marine, (2) the chromosome number of the red algae is greater than that of the Basidiomycetes, (3) nuclear division takes place in quite a different fashion, and (4) the life-cycles, while paralleling to a degree those of the Basidiomycetes, are analogous, not homologous, since the alternation of generation in the red algae, for the most part, involves two separate types of plants, one having N-chromosomes, and the other 2–N, whereas in the Basidiomycetes the cycles involve an N-chromosome phase and an N + N phase in addition to the 2–N phase which, relative to the red algae, is only momentary.

The other alternative to the red algae as the ancestral stock of the Basidiomycetes is the Ascomycetes. This hypothesis, as may be ascertained from a perusal of the more recent literature, is becoming more generally accepted, and not without good reason, since the Basidiomycetes represent a culmination of an evolutionary

trend that began very early in the evolution of the Ascomycetes, namely the increasing importance of the dikaryotic phase that is interposed between plasmogamy and caryogamy. This trend and the homologies between the crozier cells and the clamp connections, and between the ascus and the basidium as has already been pointed out by Gäumann (**1926**) and further emphasized by Rogers (**1934**) and others, seem sufficient to warrant the assumption of an ascomycetous ancestor for the Basidiomycetes.

Among the ascomycetous forms, *Ascocorticium* has been considered to represent a possible point of departure. The reasons for the choice of this genus as a starting point are admirably summed up by Rogers (**1934**) as follows: "Although there is no incontrovertible evidence that the evolution of the basidium has at any level been monophyletic, yet the numerous fundamental similarities among the various basidial types speak for their origin from an at most greatly restricted ancestral group. The complete series of basidial types being represented in resupinate forms only, it is proper to reason that the greater part of the fundamental morphological evolution has occurred at this level of development, and also to postulate the rise of the Basidiomycetes from such resupinate form as *Ascocorticium*. Further, the sterigma-spore relation, as has been shown by Buller, is morphologically and physiologically a fixed one throughout the gymnocarpous basidiomycetes; this being the case, it has either developed *de novo*—and scarcely more than once—in the course of basidial evolution, or else it represents, likely in a modified form, a biological adaptation already present in the ancestral ascomycete. The first implies development of all basidiomycetes from a single basidiomycetous ancestor; polyphyletic evolution of the group has any considerable probability only under the second hypothesis." The writer is in hearty agreement with the suggestion that the Basidiomycetes are probably monophyletic in their origin, but cannot agree that the resupinate character, even though associated with all the different basidial types, must needs point to an *Ascocorticium*-like ancestor since it seems more than likely that the resupinate character may not only develop independently among the different groups, but also that it is a secondary development and derived from the pulvinate type of fructification.

It is the writer's belief that the Uredinales or an ancestral form of this order should be considered to be the primitive Basidiomycetes. On this basis, the ascomycetous ancestor would possess the following characteristics: during the life cycle it should produce pycnidia with receptive or "Woronin hyphae," it should have one or two additional conidial forms, and possibly it should be parasitic. These qualifications are met by the Pyrenomycetes and among these the order Dothideales, in its older and more general sense, may well be suggested as a starting point, although for the purposes of the present discussion the exact allocation of the ancestral form serves no useful purpose since the issue is not where but how the Basidiomycetes arose from the Ascomycetes.

The demonstration of the nature of the pycnidium by Craigie (**1927**) suggests, and the subsequent work by Allen (**1934**), Andrus (**1931**), Hunter (**1936**) and others emphasize the remarkable parallelism between the rusts and the Ascomycetes. This parallelism is so remarkable that it cannot lightly be dismissed, as is done by those who would derive the rusts from the *Corticium* or *Tulasnella*-type of basidium. Wolf (**1935**) has shown that the pycnidium of the dothideaceous *Cymadothea* possesses receptive hyphae that are exactly homologous with those figured for the rusts. Indeed were the connections with the perfect stages not previously known, it would be difficult if not impossible to tell the ascomycetous and the uredinaceous pycnidia apart. A comparison of the mechanism of fertilization may be carried even further as a result of the recent excellent investigations of Backus (**1939**) who has shown that the "Woronin hyphae" or receptive structure of *Neurospora* may be extremely elongate and branched, and this structure may well be compared with the stomatal hyphae which are reported in the Uredinales. The exact reproduction in the rusts of the methods of fertilization, and of the structures associated with this process, in the Pyrenomycetes should serve as the strongest evidence for relationship between the two groups. If, then, it may be assumed that the pycnidia of both classes of fungi are homologous, it is only necessary to postulate that during the course of time and as a result of various environmental factors, many forms survived in which the sequence of spore types became fixed. Thus the pycnidia were produced first, and as a result

the conidial types, as exemplified by the aecidiospores and uredospores, have come to be dikaryotic bodies for the dissemination of the organism during periods favorable for growth. This hypothesis is not made without some justification, for, as already pointed out, the trend within the Ascomycetes has been towards the increase in the duration and importance of the dikaryotic stage of the life-cycle, a trend that is continued in the rusts when the spores, aecial and uredo of the present day forms, became dikaryotic. The spores thus equipped with nuclei of the opposite sex factors serve to spread the fungus more rapidly since, both factors being present, the thallus may immediately proceed to the formation of the zeugites or resistant structures with the onslaught of unfavorable conditions, thereby not only facilitating spread, but also the survival of the fungus. Thus it may fairly be stated that increased emphasis is placed on the dikaryon phase.

The comparison between the Ascomycetes and the Basidiomycetes may be pursued further and the asci may be compared with the teleutospore of the rusts. In many genera of the Inoperculate Ascomycetes, as in *Parodiopsis, Myriangium, Leptosphaeria, Mycosphaerella,* and in other more distantly related genera, there are many species of which the asci are characterized by an apical thickening of the wall and in that thickened portion there is provided a large pore-like modification of the wall which is comparable to that found in the teleutospores of *Melampsora, Uromyces* and many other genera of the Uredinales. If the asci of this type, that is, with the thickened wall and provided with an apical pore-like modification, were formed without the protection of the perithecium, mutual pressure as well as pressure exerted against the epidermis of the host would result in the formation of a palisade-like layer that would be comparable to that found in the rusts of today as exemplified by species of *Melampsora.* Presumably the hypothetical ancestral form may originally have germinated as do many of the living species of Pyrenomycetes. The asci of species of *Pleospora, Leptosphaeria, Sporormia,* and *Melanomma,* according to Ingold (**1933**), are provided with a rigid outer wall and a thin elastic inner wall. Upon germination, the pore-like modification at the apex of the asci is either greatly softened or ruptured and the inner wall then emerges through the

resulting pore with almost explosive rapidity (FIG. 1); thus the spores are protruded beyond the rigid ascus wall still within a slender membrane, a condition that foreshadows the development of the basidium of the rusts. In the Uredinales there is a delay

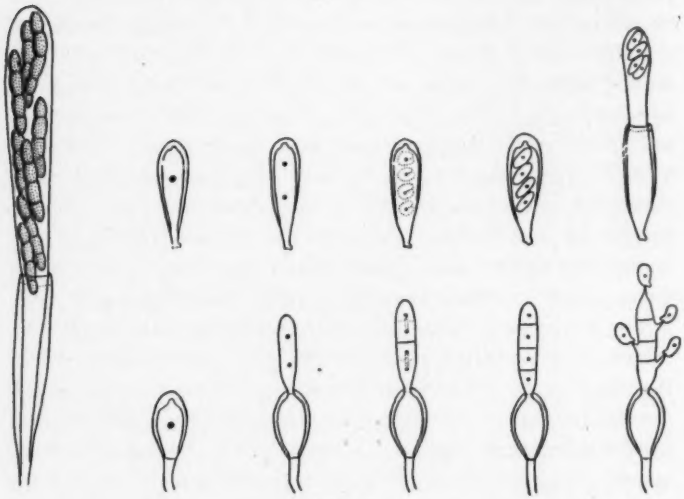

FIG. 1.

in nuclear division until the germ-tube, which is the counterpart of the thin-walled internal ascus, has grown through the pore and the nucleus and accompanying protoplasm have migrated into this structure. Nuclear division then takes place, but each nucleus, instead of becoming surrounded by a wall as in the Ascomycetes, becomes separated from the others merely by a cross-wall. Thus the same four-celled structure is found in the rust basidium as is found within the extruded inner elastic wall of the Ascomycetes, but instead of being represented by free spores, they are represented by cells.

The above derivation of the basidium seems to explain the fixity of the number of cells of the majority of the lower Basidiomycetes, namely four, and is all the more comprehensible if the derivation is from an ascomycetous ancestor in which four-sporedness had become a relatively fixed characteristic. The substitution

of cross-wall formation in the basidium for free-cell formation as in the ascus does not seem to be an insuperable barrier to accepting this method of origin. If, on the other hand, the basidium were derived from an *Ascocorticium*-like ancestor with a thin-walled ascus, it would be necessary to postulate, as Neuhoff (**1924**) does, that the homobasidial types were derived first and then during long periods necessary for the evolution of the rust types, the basidia went through stages of transformation exemplified by the genera *Gloeocystidium, Kordyanella, Iola, Cystobasidium,* and *Uromyces* to *Puccinia* which according to this system would place the Uredinales in highest place. In so deriving the group, the highest form is that in which the method of fertilization is characteristic of the Ascomycetes. If such were the line of evolution, it may be remarked that Mother Nature wasted much time and a golden opportunity to attain her ends with much less difficulty. Another objection to using the holobasidium as a starting point for the remainder of the Basidiomycetes is that it is necessary to imagine that the formation of sterigmata came about as a result of the terminal proliferation of the ascus in the manner illustrated by *Taphrina* in which Sadebeck (**1884**) found that young asci under certain conditions germinate with sprout mycelia upon which conidia were formed. However, it should be pointed out that the asci were still young and had not as yet differentiated ascospores. Under such conditions, it would therefore seem that in immature asci when subjected to a definite set of conditions that favor vegetative growth, the reproductive processes are suppressed and the vegetative phase becomes reestablished. If this is correct, then it would be difficult to derive the basidium, which is strictly a result of reproductive activity, from the ascus in this manner. In view of the fixity of the position, number, and nature of the sterigmata on the basidium, it hardly seems probable, especially in view of the fact that in *Taphrina* at least, the process of spore formation is that of budding, and not of extrusion and eventual discharge as is true for the basidiospores. In the sense of the budding by the ascus, the basidium is not a proliferative structure since, with but a few exceptions, once the basidium is formed, it contains the entire content that was formerly in the probasidium, as eventually do the spores. If the basidium were proliferative in nature, then

it should retain at least a portion of the protoplasm so that additional spores might be formed in succession. In this respect, the basidium closely agrees with the ascus.

It may be objected that the absence of clamp connection in the Uredinales prevents this order from being an ancestral group from which the other members of the Basidiomycetes may have been derived. It has not been definitely proved that clamp connections may not be present in the rusts. Even though this were conclusively proved, we have no way of demonstrating that such structures were not present in the early forms. It may be that the ancestral forms did have clamp connections, but because of the fact that the group has been intramatrical over an extremely long period the clamp connections as a consequence have been lost. A parallel to this situation exists today, for Greis (**1938**) and others have shown that in the Polyporaceae, the mycelium that is imbedded in the substratum, even though dikaryotic, does not produce clamp connections, yet once outside in the air they may be formed on the mycelium in relative abundance, thus suggesting an oxygen relation. However, a physiological explanation is not necessarily as important as it may appear to be, for an explanation may readily be found in the phylogeny of the group. If, as has been proposed above, the Uredinales are derived from a pyrenomycetous ancestor, there is no need for supposing that clamp connections have as yet developed. Crozier formation in certain members of what formerly was the order Dothideales, is not at all well developed nor is it highly developed in many of the Sphaeriales. It is not until the higher families of the Inoperculate Discomycetes that crozier formation has become well fixed, and in the higher of these groups even clamp connections may be found in the vicinity of the hymenium. Thus it may be said that crozier formation becomes important when a large number of asci result from a single or relatively few acts of fertilization. The structure therefore facilitates proliferation and the formation of numerous reproductive bodies (asci). It should be remembered that the mycelium subtending the fruiting bodies of the Inoperculate Discomycetes is haploid. In the rusts, the need for proliferation within a limited fructification is absent since dikaryotization takes place early in the life-history and as a result the proliferative service of clamp con-

nections has not yet become necessary. Since the species are parasitic, and since the substratum is relatively abundant, the need for the production of a vast number of basidiospores is not so great. Even though the host were not abundant, the dikaryotic conidia serve equally well to insure the spread of the parasite. With the shortening of the life cycle and with the change from parasitic to

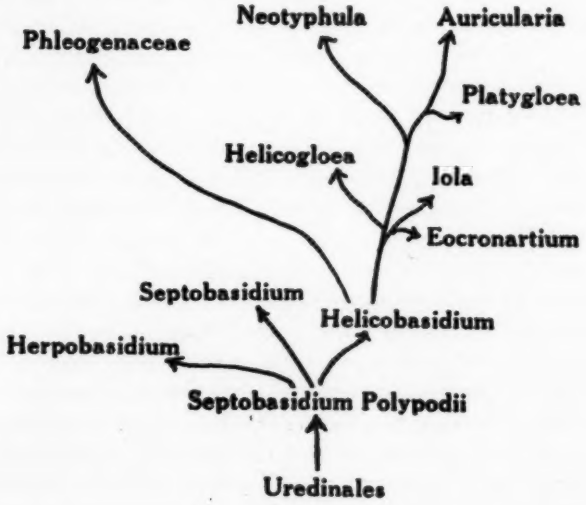

FIG. 2.

a saprophytic or symbiotic mode of life, the need for proliferation comes in since then the fruiting body becomes more extensive. Thus we find that for the first time, *Septobasidium* and *Helicobasidium,* for example, produce large numbers of probasidia over an extensive area, and in these genera, except in those species in which the tendency towards homothallism enters to confuse the picture, clamp connections are present for the first time. The absence of clamp connections, if this be true, in the Uredinales, may then be explained by stating that in regard to these structures, the rusts have reached a degree of development parallel to that found in the higher Pyrenomycetes but slightly lower than that in the Inoperculate Discomycetes.

Further consideration of the phylogenetic trends within the

Uredinales, excepting as it is relative to the origin of other groups, is superfluous here in the light of the many papers on the subject by Arthur (**1929**), Cummins (**1936**), Cunningham (**1931**), Dietel (**1938**), Faull (**1929**), Hiratsuka (**1936**), Jackson (**1931**), Mordvilko (**1926**) and others. The morphology of the basidium within the order is comparatively constant and this in spite of the fact that nuclear behavior, as a result of the trend towards homothallism, may be extremely variable. It is this variation in nuclear behavior, in addition to the shortening of the life-cycle, that makes possible the derivation of the remaining Basidiomycetes from the rusts. For convenience and brevity, only three evolutionary lines will be discussed and these in the order listed: (1) Uredinales—Auriculariales—Dacryomycetales, (2) Uredinales—Ustilaginales, and (3) Uredinales—Tremellales—Autobasidiomycetes.

UREDINALES—AURICULARIALES

In a previous paper, the writer (**1929**) in discussing *Saccoblastia intermedia* [*Helicogloea intermedia* (Linder) Baker] indicated that the Auriculariales appeared to be derived from and are not ancestral to the rusts. Since that time additional evidence has accumulated to confirm this view. Craigie's (**1927**) exposition of the nature of the pycnidium was followed by a series of papers demonstrating the method of fertilization in the rusts, and Ames (**1932, 1934**) and Drayton (**1934**), to name but two investigators, by showing that spermatization takes place among the Inoperculate Ascomycetes (here employed to include both the Pyrenomycetes and Inoperculate Discomycetes) have demonstrated the identity of the methods of fertilization in the two groups of fungi. During this period also, Jackson (**1931**) has clearly indicated that the evolutionary trends within the Uredinales all lead towards the shortening of the life-cycle, and in a later paper (**1935**) has shown that to a marked degree there is a correlation between this trend and a change from heterothallism to homothallism. There may be a question as to whether it was the change of hosts during the course of evolution or the shortening of the cycle that has led to homothallism. Whichever is the cause, it is sufficient at this point to realize that changes in nuclear behavior, in morphology, and in life-cycles has taken place and has been expressed in different

manners by different members of the group. If then the long-cycle forms are considered to be primitive, it would indeed be difficult to derive the rusts from the Auriculariales. None of the members of that order, so far as the writer is aware, possess pycnidia in their life-cycle and therefore to derive the Uredinales through the highest forms, that is to say, those forms with an abbreviated life-cycle and in which their nuclear behavior has become quite variable as a result of the trend towards homothallism, and to expect evolution to proceed backwards towards those forms with a long cycle which are considered by most Uredinologists to be the most primitive, is illogical to say the least. For this reason and for others already outlined, it becomes necessary to consider the Uredinales as the ancestral group from which sprang the Auriculariales.

As has been stated repeatedly, the Uredinales have proceeded from the complex to the simple life-cycle. During the evolutionary change, not only have spore forms dropped out in various fashions, but correlated with this to a remarkable degree, it would appear that spermatization also has tended to fall by the wayside and the pycnidia and pycnidiospores to become functionless or obsolete, thus leading to dikaryotization through hyphal fusions in the manner described by Christman (**1907**) for *Phragmidium Potentillae-canadensis,* and by other investigators for other rusts in different genera. When spermatization takes place in the higher short-cycle forms, then it is more than likely that the rôle of pycnidiospores has been taken over by the secondary sporidia produced by the basidiospore or by hyphal fusions. Whatever the mode of fertilization in the short-cycle rusts may be, the trend within the order seems to be towards the Auriculariales.

Another tendency towards the Auriculariales is shown to some degree by the genera *Cystospora* and *Tranzschelia* of the Pucciniaceae, and to a conspicuous degree by *Goplana mirabilis* of the Coleosporeae—namely the extramatrical production of the probasidium and the tendency towards exoparasitism. It is not beyond the realm of probability to consider that these forms of today merely duplicate what has taken place in the geologic past, and accordingly the Auriculariales as represented by *Septobasidium Polypodii* Couch (**1929**) and by *Uredinella coccidiophaga* Couch

(**1937**) may be considered to be derived from ancestors which at an early date became extramatrical. With the exception of *S. Polypodii* and another undescribed species that has been observed by the writer, the genus *Septobasidium* early changed from an external parasite on the host plant to a parasitic symbiont of scale insects, a change that has become a relatively fixed characteristic of the genus. However, from the *S. Polypodii* type of ancestor there has also arisen a line that leads directly to *Helicobasidium* and *Cystobasidium* and it is characterized by the increasingly saprophytic mode of living. Within the genus *Helicobasidium,* just as in *Septobasidium,* there is a tendency towards homothallism or parthenogamy, if we compare it with the parallel situation illustrated by the Endomycetales and Saccharomycetales, and at the same time there is a gradual loss of the probasidium. These two characteristics do not necessarily go hand in hand since there is also an evident loss in the development of the probasidium in the heterothallic species which, it would seem, gave rise to the higher Auriculariaceae, as is indicated in the accompanying phylogenetic scheme. The Phleogenaceae would appear to have been derived from an ancestral species in *Helicobasidium* that had already lost the probasidium and at the same time has become more or less saprophytic. On the other hand, *Iola,* as shown by the accompanying chart, still exhibits the probasidium and may be considered to be parasitic. The genus on the one side seems to be ancestral to *Eocronartium* which is parasitic on mosses, and on the other to *Helicogloea* which to a degree still produces probasidia but at the same time is saprophytic or in some instances may be parasitic. This latter genus appears to be a side branch, but possibly it may have given rise to *Platygloea* and eventually to *Neotyphula* and *Auricularia* which are mostly saprophytic forms that have developed more complex fruiting bodies and at the same time have lost the definite probasidial structures that are present in the lower groups.

Returning again to the *Septobasidium Polypodii* ancestral type, it would appear that from this has arisen the monotypic genus *Herpobasidium,* a genus which, although parasitic, nevertheless has lost the probasidium and has proceeded towards the homothallic condition described by Jackson (**1935**). With this tendency to-

wards homothallism, there have been introduced irregularities in nuclear behavior and at the same time changes in the structure of the basidium with the result that that structure resembles the basidium of *Dacryomyces*. It seems doubtful, however, that *Herpobasidium* has given rise to any further groups, although such a possibility cannot be definitely excluded. The reasons for considering *Herpobasidium* to be the end of an evolutionary line will be discussed in a following paragraph. For the present it will suffice to sum up the origin and evolution within the Auriculariales by pointing out in the accompanying diagram that those forms with the characteristically coiled haustoria, such as are present in some of the Uredinales and are well illustrated in the genus *Septobasidium*, are closely allied, that they are arranged according to the decreasing importance of the probasidium, and finally that the parasitic forms are grouped together and are considered to be more primitive than the saprophytic forms.

Before entering a discussion of the origin of the Dacryomycetales, mention should be made of the part played by parasitism or saprophytism in evolution, since much has been written upon this subject, and for the most part there has been a tendency to look upon the parasitic mode of living as something fixed and unchanging. For the present day members of the Peronosporales and the Uredinales, this seems to hold. However, it should be kept in mind that these present day obligate forms are the result of long ages of selection and evolution towards specialization. There are other groups which also show specialization of parasitism as is exemplified by the genera *Sclerotinia* and *Mycosphaerella*, to name but two, and yet these forms may readily be grown in culture. The same is true of the Ustilaginales. Even in the rusts there is evidence that there may be a tendency away from strict parasitism, since the writer has observed in the field that some species continue to produce fruiting bodies after the host substratum has passed a stage that would most liberally be termed senescence. Thus teleutospores of *Uromyces Fabae* have been observed to be formed in the autumn on stems of *Vicia cracca* which had apparently been killed in late summer by the uredo stage. If this can happen today after the forms have gone through long ages of host specialization, then why could not forms become weakly parasitic or

saprophytic during a much earlier period when the characteristics had become less well fixed and when the species were in a young and more "fluid" state? Newton (**1939**) has stated that "Certain pathogenic characters (infection types) have proved dominant over others, and the distribution of the characters in F_2 and subsequent generations has suggested Mendelian inheritance." If parasitism is a result of Mendelian factors and also, as was suggested in the same paper, may be influenced by the cytoplasm, then the chances are fair that during the ages, mutations could have occurred which gave rise to weakly parasitic or even saprophytic species.

DACRYOMYCETALES

The relationship of the Dacryomycetales to the Auriculariales is indeed dubious and it is considered here only because in the genus *Herpobasidium* there is found one clue as to the manner of origin of the non-septate basidium. Reference to Lind's paper (**1908**) or to that of Jackson (**1935**), will demonstrate that in *Herpobasidium* the basidium is two-celled and that the two sterigmata often parallel each other. Thus if the basidium were to lose its single septum, the forked basidium would result. According to Dangeard's (**1895**) interpretation of the cytology of the basidium, such a derivation would be acceptable since then it would be necessary to postulate only the loss of the basidial septum and the survival of but a single nuclear division. However, Juel (**1898**) studying the same species, *Dacryomyces deliquescens,* found that the fusion nucleus, instead of dividing but a single time, divided twice to produce four daughter nuclei of which two migrated into the spores. These results of Juel have further been substantiated by the work of Gilbert (**1921**) who studied an undetermined species of *Dacryomyces,* and by Bodman (**1938**) who investigated the cytology of *Guepinia Spathularia.* By correlating observations made on the Uredinales in which the four-nucleate basidium is considered to be the more primitive, it would be impossible to derive the four-nucleate dacryomycetaceous basidium from the binucleate *Herpobasidium* type which has already passed through stages of reduction to reach this condition. It therefore is necessary to look elsewhere for the ancestral type. Since the basidium is four nucleate in those dacryomycetaceous forms that have been

studied, since binucleate conidia comparable to aeciospores are often present, and also since the characteristic yellow-orange pigment that is common to both groups is present, the relationship to the Uredinales seems closer than the morphology of the basidium would at first glance lead one to suspect. If on these grounds the

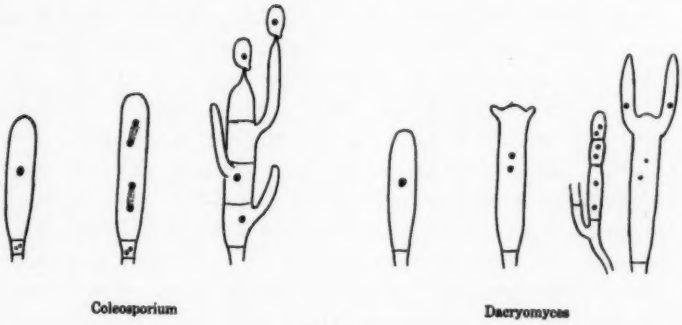

FIG. 3.

relationship with the rusts is as close as it would appear to be, the affinity would probably be not with the Pucciniaceae because of the well developed probasidium in that family, but more likely would be with the Coleosporeae in which the probasidium is directly converted into a basidium (FIG. 3). To derive the Dacryomycetales from this source, it would be necessary to conjecture that the progenitors were forms that had become saprophytic in their mode of life, and that the sorus had become imbedded in a more copious gelatinous matrix. At the same time, as a result of variations in nuclear behavior similar to those already mentioned, the septa have been lost, although the double division of the fusion nucleus still persists,[2] even though but two of the four daughter nuclei migrate into the sterigmata and eventually into the basidiospores, while the other two remain behind in the basidium and degenerate. According to this theory, the sterigmata are not special organs to be labelled epibasidia but in fact are exactly homologous with those of the Uredinales or the Auriculariales, and

[2] It is possible that Dangeard investigated a species other than *D. deliquescens* or else a homothallic strain of that species, the fusion nucleus of which failed to divide twice.

like those of the latter group they vary in length in proportion to the depth to which they are buried in the gelatinous matrix. That their number is constantly two to a basidium is explained by the fact that the basidium has lost its septa and that nuclear behavior has become so modified that only two of the four daughter nuclei are functional.

It is questionable whether the order Dacryomycetales has given rise to other groups of the higher Basidiomycetes since the nuclear behavior and the structure of the basidium appear to set the order apart as a distinct group that ends an evolutionary line. Accordingly, although the basidium is homobasidial in structure, it is phragmobasidial in nature. Thus the order finds a logical place among the Protobasidiomycetes. Within the order, further evolution has proceeded in two directions, the first from the pustulate forms of *Dacryomyces* to the resupinate genus *Ceracea* and then to *Arrhytidia* which may be more or less resupinate but with free margins, or more or less saucer-shaped. The other line of evolutions is towards the stipitate fruiting bodies and begins with *Dacryomyces,* goes through *Guepinia* and *Dacryomitra* on the one hand, and *Calocera* on the other. Thus the order of development closely parallels that of the Auriculariales and, in fact, the other groups of the Basidiomycetes in which there is any considerable degree of variation in the shape and size of the basidiocarp.

USTILAGINALES

For the origin of the Ustilaginales it is also necessary to turn back to the Uredinales. It again is evident that the group has resulted from the shortening of the life-cycle of the rusts, but in this instance it is necessary to look for the place of origin not in the short cycle forms which produce typical teleutospores, but rather in the endo-forms in which the aeciospores have taken over the function of the probasidium. Since there is no reason why teleutospores should not be dropped out of the rust life-cycle just as have the aeciospores and uredospores, the chlamydospores of the smuts are considered to be identical in nature with the aeciospores of the endo-forms, and like them to germinate by means of a characteristic basidium. Evidence for the derivation of the smuts from the rusts in this manner is furnished by species similar to

Endophyllum Euphorbiae-sylvaticae, in which Moreau (**1919**) has shown that the paired nuclei, without fusing in the probasidium, pass into the young basidium, divide and become separated by septa to form a four-celled basidium. The daughter nuclei migrate into the basidiospores and then divide or else they may divide prior to their migration, but in either case the entire content of each basidial cell passes into the spores. It will be recalled that

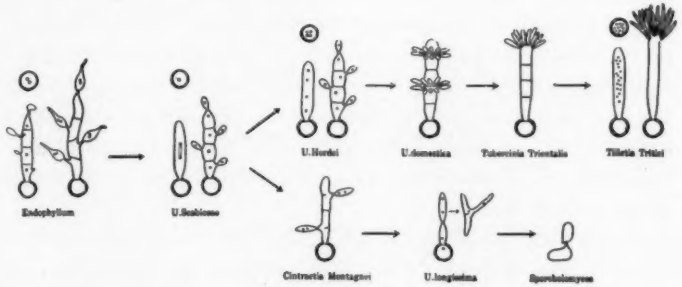

FIG. 4.

in certain of the Ustilaginales the nuclei fuse prior to the formation of the basidium, and also that during the process of spore formation, the nucleus in each basidial cell divides, one daughter nucleus passing into the spore while the other remains behind to divide further and produce other spores. In these two main aspects, the forms represented by species of *Endophyllum* and present day Ustilaginales are distinct, yet it is not difficult to imagine an *Endophyllum*-like ancestor in which caryogamy takes place prior to basidial formation and then when the basidium has formed, the nuclei divide in the basidial cells, one of which resulting nuclei passes into the spore while the other remains behind to carry on in a manner identical with the present day smuts. Such a form would be exactly intermediate between the rusts and the smuts, and in view of the great diversity of nuclear behavior in the two groups, it is not difficult to imagine such a form as existing today or as having existed in the past. Aside from the resemblance between the aecial type of teleutospore and the so-called chlamydospore, there is also a similarity in methods of fertilization. According to Hanna (**1929**), although infection of the host by single

germinating spores takes place, there is no formation of chlamydospores (or probasidia) until there is fusion between spores or between mycelium of opposite sex potential, a fact that would be expected according to the hypothesis that when spermatization disappears, plasmogamy takes its place. The fusion of secondary basidiospores or sporidia seems to be a later development that has arisen since the Ustilaginales were derived from the Uredinales, and the trend towards fusion of the spores on the basidium not only represents an advancement of the position in the life-cycle of dikaryotization but also represents a more efficient method for assuring the dissemination of the species, since it has resulted in the insurance that nuclei of opposite sex potential meet in a less accidental fashion than would be true if the spores germinated individually and relied on chance for mycelia of opposite sex potentiality to meet.

On the assumption that the Ustilaginaceae were derived from an endo-form of the Pucciniaceae as a result of the aeciospores taking over the functions of the teleutospore and also as a result of a slight change in nuclear behavior, the Ustilaginaceae would appear to be more primitive than the Tilletiaceae. Within the former family, evolution appears to have proceeded in two lines: towards the formation of a coenocytic basidium and the apical production of basidiospores, and in the other direction towards the reduction of the basidium.

Following the first line of evolution within the Ustilaginaceae, there is an evident trend from the typical ustilaginaceous basidium in which, like those of the rusts, the first and second division of the fusion nucleus takes place within the basidium, and the spores are produced one at a time on each basidial cell. This is exemplified by *Ustilago Scabiosae* which was studied by Harper (**1898**). The next step is shown by *Ustilago Hordei* in which both the first and second divisions of the fusion nuclei take place, not in the basidium, but in the probasidium, thus advancing the place and time of meiosis, although basidiospores continue to be produced singly on each basidial cell. Slightly more advanced is *Ustilago domestica* since, instead of producing the basidiospores singly and successively on each cell, the spores are formed in greater numbers and almost simultaneously on the basidial cells as is shown in fig-

ure 4. In addition to this, the spores are definitely localized so that a ring of spores is produced at the basal end of the outermost cell, the apical end of the second cell, the basal end of the third cell and the apical end of the fourth cell in such a manner that fusions, since sex segregation has taken place in such a manner that nuclei of opposite sex factors are in adjacent cells, may take place readily between opposing spores. The next step in advancement, shown by *Tubercinia Trientalis* of the Tilletiaceae, leads to the localization of the basidiospores to the apex of the basidium which is still septate. The final stage in the evolutionary line is reached in *Tilletia Tritici* where most of the divisions of the fusion nucleus take place in the probasidium with a few occasionally occurring in the basidium which is usually non-septate and proceeds directly and efficiently to acrogenous basidiospore formation. Fusion between the spores occurs while they still remain attached to the basidium. As is summarized in figure 4, the trend has been from the septate to the non-septate basidium, from a single division following meiosis to several, from division in the basidium to division of the fusion nucleus in the probasidium, from pleurogenous spore formation to acrogenous, and finally from 4- to many-sporedness, with fusions taking place between compatible basidiospores.

The second line of evolution within the Ustilaginales is towards the reduction of the basidium and a decrease in the number of spores. In this series the reduction of the basidium seems to be the consequence of plasmogamy between adjacent cells whereby both meiosis and dikaryotization have been compressed into the single organ and this has led to its degeneration. Thus in *Ustilago Scabiosae* and in many other species, the spores are freed before conjugation, whereas in *Cintractia Montagnei* (FIG. 4) there are fusions between the adjacent cells by means of clamp connections, and on each of the two clamps a single basidiospore is formed into which pass the + and — nuclei. Although as a result of this method of dikaryotization the spores are equipped with nuclei of opposite sex potentialities and are able to reproduce the species after infecting the host, nevertheless it is the first step in the reduction of the basidium since now it is only able to produce two spores and therefore is only the equivalent of a two-celled

basidium. *Ustilago nuda* becomes more reduced since there is a delay in the formation of basidiospores after plasmogamy, but this reduction is carried by *Ustilago longissima* var. *macrospora* to a stage which simulates apogamy in which only one binucleate spore is formed at a time. The two nuclei of opposite sex factors are separated by a septum but these then come together by means of a clamp connection and pair, and the germ tube which is later formed produces sporidia that fuse normally. The last stage of reduction is reached in *Sporobolomyces* where there is apparently a complete loss of sex. Thus this line of evolution closely parallels that found in the Ascomycetes as illustrated by the Endomycetales-Saccharomycetales series postulated by Guillermond (**1902**), and apparently the same factors are involved.

TREMELLALES—AUTOBASIDIOMYCETES

Just as the Auriculariales and the Ustilaginales appear to have had their origin in the Uredinales, so too does it seem more than likely that the Tremellales trace back to the same source. The septate basidium immediately marks this order as relatively primitive, as does also the germination of the basidiospores by repetition. The best evidence for the manner in which the Tremellales originated is furnished by Weir (**1912**) who reported that not all the probasidia of *Coleosporium Pulsatillae* germinate to form transversely septate basidia, but that some of them germinate and become cruciately divided and thus are homologous with the basidium of the Tremellales. Hence if the spore-bearing projection from each cell of the transversely septate basidium is labelled a sterigma, then logically the same projections from the cruciately divided basidia must fall in the same category and the term epibasidium becomes superfluous. If the Tremellales are derived from an ancestral uredinaceous form that was characterized by a change from the stichobasidial orientation of the nucleus to the chiastobasidial, it may be assumed that these forms have become saprophytic during the passage of time and coincidently with this change there has been a great increase in gel production over that found in the coleosporiaceous ancestor. Just as in the Dacryomycetales and the Auriculariales, there are present in this group conidia which are at first binucleate and, although the spores may

become uninucleate through subsequent septation, they may well represent a remnant of the habit of forming aeciospores.

In thus deriving the Tremellales from the Coleosporeae, it would seem that the most primitive form would be pustulate. Since the basidiospores of *Tremella* produce sporidia closely resembling those of the rusts, it is considered the most primitive genus and *Exidia* with the sickle-shaped sporidia is derived from this genus.

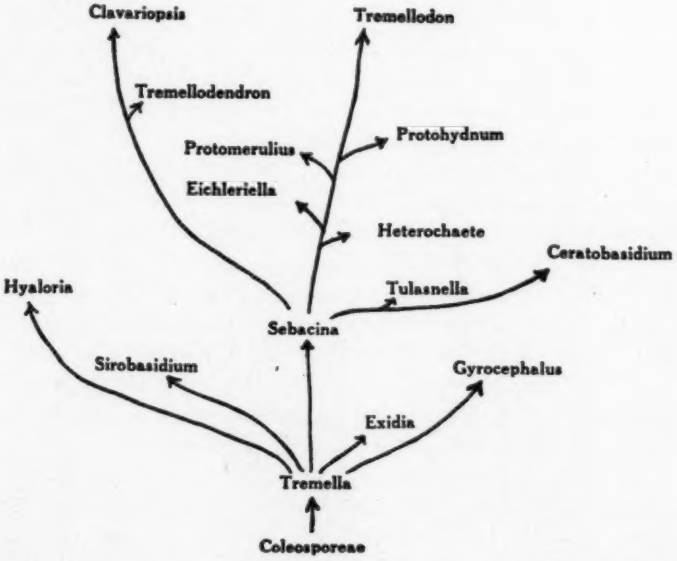

FIG. 5.

From *Tremella* also, through the assumption of the resupinate habit of growth, comes *Sebacina* which in turn gives rise to three evolutionary lines: (1) *Sebacina—Tremellodendron—Clavariopsis,* (2) *Sebacina—Heterochaete—Eichleriella—Protomerulius—Protohydnum—Tremellodon,* (3) *Sebacina—Tulasnella* as shown in figure 5. The Sirobasidiaceae are derived from *Tremella* also and represent a line that has resulted from the basipetalous proliferation of basidia by the efficient utilization of the dikaryotic cells of the subtending mycelium. The position of the Hyaloriaceae is less obvious since the fructification is angiocarpous. However, it seems

that this monotypic family may be derived from a *Tremella*-like ancestor through the production of a more or less stromatic base surmounted by the hymenium which is penetrated by elongate, sterile, paraphysoid, gel secreting hyphae among which the basidia are scattered. In general, the evolutionary lines in this order follow closely those in other groups already discussed.

At this point it seems desirable to examine the Tulasnellaceae more carefully since much has been made of *Tulasnella* as a primitive genus among the Basidiomycetes, and as a connecting link between some ascomycetous ancestor and the Autobasidiomycetes and the Tremellales. It appears that the Tulasnellaceae have received more attention in this connection than the family justly deserves. If the Tremellaceae are briefly surveyed, it will be found that within the family there is considerable variation in the septation of the basidium, both as regards to orientation and to number of septa (Coker **1920**; Whelden **1934, 1935**). Since one or two or even three of the longitudinal septa (Whelden **1935**) may be lost, there should be no reason why all of the septa may not, and when this happens the resulting basidium is similar to that described by Rogers (**1935**) for *Ceratobasidium* or for *Tulasnella* (**1932**). Furthermore, if the survey is continued, it will be found that germination by repetition plays a very important rôle in the group. When the basidiospores germinate, they do so by producing one or more germ tubes, each of which produces one, or, according to Brefeld (**1888**), in the case of *Exidia repanda* more than one sporidium. When more than one sporidium is formed, it is obvious that there must be one or more divisions of the nucleus in the basidiospore. During the course of evolution, one of these divisions of the nuclei is advanced in time and place, coming to take place in the sterigmata. Whether or not this swelling of the sterigma to spore-like proportions is a result of nuclear activity or whether the swollen sterigmata in the past have served as a temporary resting point for those species which grew in cold weather or encountered drouths during the growing season, cannot satisfactorily be explained without more information in regard to the life history and biology of the forms. It is, however, significant that there is a transition to or from the conspicuously inflated sterigmata to the less inflated sterigmata characteristic of *Cerato-*

basidium or to a non-septate tremellaceous basidium. It is also significant that the spore types exactly parallel those found on the one side in *Sebacina* and on the other, those found among the primitive members of the Corticiaceae. This parallelism of spore

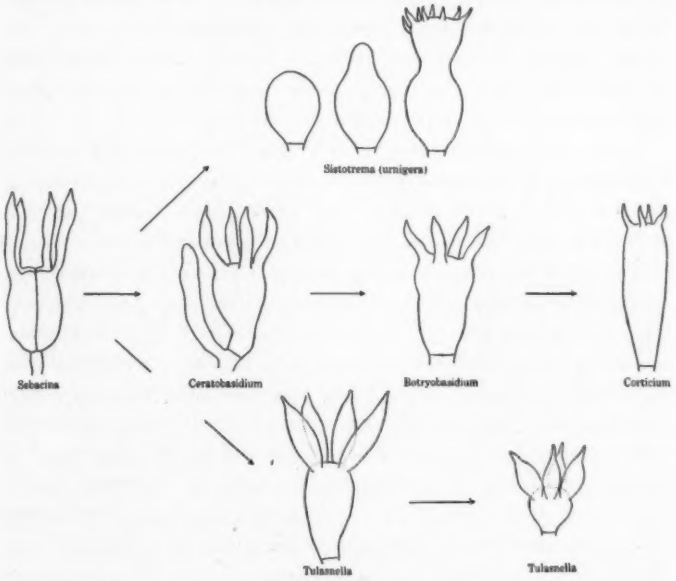

FIG. 6.

types seems to indicate a definite relation between the Tulasnellaceae and the other two groups and to suggest that it represents an end line that is intermediate between the Tremellaceae and *Ceratobasidium* in its origin, with those species characterized by conidium-like sterigmata representing the highest development. At all events, the basidium with the conidium-like sterigmata scarcely represents a primitive type derived from an ascus as Rogers (**1932**) intimates when he observes that "The sporoid characters of the *Tulasnella* epibasidium strongly suggest its evolution from the ascospores; consequently the epibasidium may be taken as the true homologue among the basidiomycetes of the ascospore, and the basidiospores as the homologues rather of an ascospore germination-conidium." So far as the writer is aware, there are no Asco-

mycetes in which the nuclei divide in the ascus in the same manner as do the nuclei of the Tremellaceae or Tulasnellaceae. The definitely chiastobasidial orientation near or somewhat below the apex of the tremellaceous basidium immediately characterizes the two basidiomycetous families and would appear to make impossible the derivation of tulasnellaceous basidium directly from an ascomycetous antecedent. For this reason it is more logical to look on the Tulasnellaceae as only secondarily derived from the Ascomycetes by way of the rusts and the Tremellales.

The Corticiaceae have been derived from the Tremellales in much the same manner as the Tulasnellaceae—by the loss of septation in the basidium. The transition, in this instance, is more clear-cut and direct since there are no secondary characters, such as are introduced by the inflated sterigmata, to obscure the relationship. As is shown in figure 6, the basidium of *Ceratobasidium* is essentially a mirror image of that of *Sebacina,* lacking only the internal septation. Furthermore the spores germinate by repetition and, as stated above, the spores show the same variation in morphology. For these various reasons, *Ceratobasidium* as described by Rogers (**1935**) therefore makes an ideal connecting genus almost exactly intermediate between the *Sebacina*-like ancestor and *Botryobasidium.* This latter genus also is in part characterized by a few species of which the basidiospores germinate by repetition, and differs from *Ceratobasidium* only in that the size of the sterigmata in proportion to the basidium is somewhat reduced. Through further reduction in the proportion of sterigmata to the basidium, and through elongation of the basidium itself, it is a simple matter to derive the *Corticium*-type of basidium which is found throughout the majority of the Corticiaceae, with the exception of those forms characterized by the *Urnigera*-type of basidium. This type appears to trace back directly to the tremellaceous basidium for in its early stage of development the probasidium is a subsphaerical body which then elongates by a relatively narrow neck that later expands and bears four to eight slender sterigmata around the apex. Before any final disposition in a phylogenetic scheme may be made, however, it would be best to know considerably more about the nuclear behavior of these forms. For the present it must suffice to hazard a guess as to its position and to point out that this type

of basidium appears to be the basis of a definite line of evolution that leads into a number of the higher forms represented in the Hydnaceae and Polyporaceae, and in part shows an evolutionary history that runs side by side with the *Corticium*-type of basidium. Thus, within the Corticiaceae are developed the three main types of basidia to be found in the higher families of the class, and these must be taken into consideration in any discussion of their classification or phylogeny.

SUMMARY

By excluding exceptional and isolated forms of the Basidiomycetes, it is possible to observe that there has been a general trend that has led from the Uredinales to the higher groups of the Basidiomycetes. This evolution has resulted from changes in life-cycles, from changes in time and position of nuclear divisions, and from changes in mode of living. It is possible to postulate that these variations have, to a great extent, resulted during geologic ages from slight differences in genetic characters that have been selected and perhaps continuously modified by environmental factors. As a result we are today dealing with forms, some of which have kept several primitive characters, but at the same time show others that are more advanced.

During the course of evolution of the Basidiomycetes, the haploid phase has become less and less important while the dikaryon stage has become dominant, and with this change, the process of spermatization has given way to plasmogamy. The basidium, once established, is essentially the same throughout the class but has undergone simplification as the result of loss of septation. Also, this same structure in the higher forms has taken over the functions of the probasidium so that it has become the locus of both caryogamy and meiosis. Because of the relatively gradual changes, and because of the essential unity in structure and function of this organ, the terminology relative to the basidium can be greatly reduced and a return may be made to the simple descriptive terms that were in vogue prior to the present age of ultra-refinement of definitions.

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THREE NEW HYPHOMYCETES PREYING ON FREE-LIVING TERRICOLOUS NEMATODES

CHARLES DRECHSLER

(WITH 3 FIGURES)

In an earlier paper (**8**) was given a comparative account of 18 interrelated mucedinaceous fungi found to subsist habitually on nematodes that they capture by means of vegetative parts variously adapted for prehension. These fungi had come to light in agar cultures started from discolored rootlets or from other affected vegetable materials, and later often further planted with pinches of friable leaf mold. Similar cultures prepared as circumstances permitted during the last few years have revealed 3 additional mucedinaceous forms obviously belonging in the same series as those previously discussed, and like them observed subsisting by the capture of free-living terricolous eelworms. As the forms appear not to have been recorded hitherto in mycological literature, they are described herein as species new to science.

A SECOND SPECIES OF DACTYLELLA WITH CONSTRICTING RINGS

A fungus more similar to *Dactylella bembicodes* Drechsl. than to any other known predaceous form appeared in numerous maize-meal-agar plate cultures that, after serving in the isolation of various species of *Pythium* from diseased plant tissues, had received, in addition to material put on them originally, some pinches of friable leaf mold collected near Butternut, Wis., in September 1938. The mycelial hyphae of the mucedinous species in question invariably grew out from the deposits of forest detritus to ramify rather sparsely through the adjacent substratum. On the hyphae were borne at intervals three-celled predaceous rings closely resembling the constricting rings produced by *D. bembicodes, Arthrobotrys dactyloides* Drechsl., *Dactylaria brochopaga* Drechsl., and *Trichothecium polybrochum* Drechsl., the general similarity in cel-

lular make-up being clearly revealed in specimens jostled into nearly horizontal positions by passing nematodes (FIG. 1, *A, a; B, a, b; C, a, b; D*). In such positions the three component cells usually display a median thickening that bulges toward the center of the ring. As is evident even when the rings are examined in their normal positions at approximately right angles to the parent filament (FIG. 1, *E; F; G, a; I, a, b; J, a*), the supporting stalk here, while also consisting regularly of two cells, is perceptibly longer than in any of the four other species with constricting organs. The incidental difference in structural detail does not make for any difference in operation; for the rings of the present fungus, like those of the constricting species previously described, capture intruding nematodes by abrupt contraction and swelling of their component cells, which then send haustorial filaments into the interior of the disabled prey to appropriate its fleshy contents (FIG. 1, *G, b; H; I, c; J, d*). Likewise, as in the other constricting species, closed rings holding no prey are to be seen near captured animals, their closure having apparently resulted from proximity to captured eelworms (FIG. 1, *J, b, c*).

Since captured eelworms lash about violently in their efforts to free themselves, it is not unnatural to presume that the stimulus effective in the unprofitable closure of constricting rings within the orbit of their struggles may be one of mechanical irritation. An instance of such closure incidentally shown in a figure of *Dactylaria brochopaga* (**8**: p. 515, fig. 13, *D, e*) was accordingly explained (**8**: p. 549) as having apparently been brought about by lashings from two specimens of *Diploscapter coronatus* (Cobb) Cobb that were found captured in rings nearby. Experimental evidence that a tactile stimulus is generally operative in closure of constricting rings has recently been supplied by Comandon and de Fonbrune (**3, 4**), who worked mainly with *D. brochopaga* and in lesser measure with *Dactylella bembicodes*. On gently irritating with a microneedle any one of the 3 arcuate cells on its inner or central side, they obtained motion pictures that recorded pronounced centripetal dilation of the irritated cell ensuing in the course of .1 second, followed a fraction of a second later by similar abrupt dilation of the other 2 cells; the intruding needle being thereupon found engaged so firmly that it could not easily be withdrawn.

However, Couch (**5**), working with *D. bembicodes,* found that movement of a fine glass rod on the inner surface of the open predaceous ring resulted in only slight swelling, and concluded therefore that mechanical irritation plays a very small, if any, part in closure of the loops. Heat, whether supplied from a hot scalpel or in water having a temperature of 33° to 75° C., he found consistently effective in springing the traps; though, as was recognized by him, a thermal stimulus can hardly be postulated in the normal entrapment of cold-blooded animals.

The divergence between Couch's findings and those of Comandon and de Fonbrune suggests that further inquiry into the physiology of the constricting ring—assuredly one of the most remarkable of plant structures—might not be misdirected. Occasion may be taken to explain in this connection that for lack of a micromanipulator my own observations on the closure of the strangulating organs have been limited so far to three instances of capture; two of these instances having occurred in cultures of *Arthrobotrys dactyloides,* the other in a culture of *Dactylaria brochopaga.* The abrupt initial closure, whereby the animal was held fast, did not seem in these few instances to effect constriction in definitive measure. Appearances indicated rather that in the region where the captured nematode was encircled its body had been squeezed to approximately one-fourth of its normal compass, so that in the beginning musculature and organs, though rather severely compressed, were by no means severed. During a period of approximately 30 minutes further constriction appeared to ensue, bringing about injury to internal parts of the animal, and consequently causing its disablement. Strangulation of prey in constricting rings was therefore considered as taking place, in some measure, progressively. Since the heavy musculature, especially of the larger sturdier nematodes, might well offer appreciable resistance to compression until its tone has become impaired by excessive exertion, partly progressive strangulation may, indeed, not be wholly at variance with complete initial closure of the predaceous ring under experimental conditions where, in the absence of a sizeable, reluctantly yielding animal body, closure is unopposed.

In an earlier paper (**6**) I recorded my failure to see any adhesive substance present on the constricting rings of the four fungi

then known to produce such predaceous organs. Rather unexpectedly some evidence of a glutinous secretion was found in examining a culture of the fungus under consideration. A completely closed ring in which no nematode had been captured was found connected with the head of a living nematode by a delicate filament of an extremely elastic substance. The animal repeatedly stretched this filament to a length of approximately 10 μ, then being apparently unable to increase its pull, it held the filament under tension for a few seconds before relaxing. On cessation of the strain, the animal's head was promptly drawn back close to the swollen ring, through contraction of the elastic tether to a length of about 1 μ. After several seconds of rest the nematode again stretched its leash with the same futile outcome as before. The effort was repeated some hundreds of times in the course of an hour. The curious gymnastic exercise appeared of little significance, except in showing vividly that an elastic adhesive substance was somehow present on the inner surface of the ring. As adhesive material would seem superfluous for detaining eelworms caught in a swollen ring, it may conceivably function in ordinary instances of capture as part of the mechanism that springs the annular trap.

When amply provided with nourishment from captured nematodes, the mycelium of the fungus gives rise to scattered erect conidiophores, each bearing a single terminal conidium (FIG. 1, *K, L, M*). This asexual reproductive apparatus reveals morphological features distinctive of the species. While in all congeneric predaceous forms previously described the conidiophore tapers continuously from base to apex, in the present fungus it expands at the apex into a knob-like termination (FIG. 1, *N, O*). Contrary to expectations that might be entertained under the circumstances, attachment of the conidium to this distended apex (FIG. 1, *K, L, M*) is not appreciably wider than in related species where no terminal modification is present. After disarticulation of the conidium its base does not show the somewhat convexly rounded truncate outline usual in related forms, but commonly retains a noticeably concave profile with rather sharp demarcation between peripheral wall and basal membrane (FIG. 1, *P, a–s, v*). This aberrance in proximal outline of the asexual spore may possibly be traceable to a somewhat different manner of conidial abjunction

from that prevailing in other species; a close juxtaposition of two septa observable at the base of some spores (FIG. 1, *P, t, u*) suggesting that abjunction may be accomplished here through separation of two distinct partitions rather than by median splitting of a single partition. Some little encouragement for such an interpretation is supplied in the fact that the conidia regularly contain only two septa, which delimit a small basal cell and a small apical cell from a large ventricose median cell; whereas the conidia of similar conformation in *Dactylella bembicodes* and *Dactylaria thaumasia* Drechsl. regularly contain three septa, spaced so as to delimit two rather small cells below and a small apical cell above a large ventricose penultimate cell. It must be admitted, of course, that the presence of a concave basal outline in the three-septate conidia occasionally produced by the form under discussion (FIG. 1, *P, v*), and the absence of such an outline in occasional two-septate spores of *D. bembicodes, D. thaumasia,* and *Arthrobotrys dactyloides,* argue somewhat against the suggested interpretation.

Uniseptate conidia (FIG. 1, *P, r, s*) are somewhat more frequently observed in cultures of the fungus than triseptate specimens, though their maturity and even more the maturity of undersized conidia devoid of cross-walls (FIG. 1, *P, q*) is naturally subject to doubt. The biseptate condition, in any case, predominates so strongly that it must be considered characteristic of the species. With respect to their main dimensions the conidia show rather moderate ranges of variation. The relevant metric data included in the diagnosis were derived from 100 measurements of biseptate conidia selected at random in equal numbers from nematode-infested cultures and from pure cultures showing abundant sporulation. These measurements gave a distribution of values for length, expressed to the nearest micron, as follows: 28 μ, 2; 29 μ, 3; 30 μ, 2; 31 μ, 3; 32 μ, 11; 33 μ, 19; 34 μ, 16; 35 μ, 21; 36 μ, 7; 37 μ, 11; 38 μ, 4; 39 μ, 1; and a distribution of values for width expressed to the nearest micron, as follows: 15 μ, 1; 16 μ, 1; 17 μ, 3; 18 μ, 9; 19 μ, 26; 20 μ, 31; 21 μ, 14; 22 μ, 11; 23 μ, 3; 24 μ, 1.

After a conidiophore has served its primary function it frequently declines to the substratum, and gives rise, often from one of its basal cells, to a secondary conidiophore (FIG. 1, *O*) whereon

another conidium is borne. Instances of somewhat different repetitional development are found occasionally when conidia, after falling on a stale substratum, germinate by the production of a delicate, erect conidiophore (FIG. 1, *Q*) that in due course bears a secondary conidium.

On fresh agar media the conidia germinate promptly by the more commonplace production of germ hyphae, which readily grow out to form extensive mycelia. Pure cultures of the fungus, free of bacterial contamination, have been obtained conveniently by removing newly formed asexual spores from the tall hyphae supporting them, to tubes of sterile maizemeal agar; the removal being accomplished by means of slabs of a sterile agar medium held on a flamed platinum spatula. In these pure cultures tall conidiophores and biseptate ventricose conidia, much like those produced by predaceous mycelia, were always formed abundantly, often with a relatively small number of hyaline conidioid bodies apparently not corresponding to any structures hitherto observed in related species. Apparently the bodies in question are never borne on tall conidiophores of the same type as those bearing the ventricose conidia, but instead are produced singly on short, erect, slightly tapering, hyaline branches, often once or twice septate, and usually measuring 10 to 20 μ in length, 3 μ in width at the base, and about 1.5 μ in width at the apex (FIG. 1, *R–U*). They are mostly of a more or less cylindrical shape, bluntly rounded at the apex and usually tapering somewhat toward the basal end (FIG. 1, *R–U; V, a–h*). They commonly measure 20 to 40 μ in length and 6 to 8 μ in width. Although occasional specimens, presumably representing early stages of development, contain vacuolate protoplasm (FIG. 1, *V, f*) not markedly different from that of young conidia, most of the bodies (FIG. 1, *V, a–e, g, h*) are largely filled with globules of nearly uniform size that appear arranged in transverse layers somewhat like bullets in canister shot. Many of the bodies (FIG. 1, *R; S; T; V, h*), including especially the shorter ones, appear almost certainly to be continuous; others show vague indications of an approximately median septum (FIG. 1, *U; V, a–e, g*), though owing to the difficult optical conditions associated with the globulose internal structure I have so far not been able to satisfy myself that a cross-wall is actually present.

The production of the elongated spore-like bodies in addition to large ventricose conidia might possibly be considered expressive of a tendency toward dimorphism comparable yet opposite to the tendency toward conidial dimorphism in *Arthrobotrys dactyloides;* for with respect to dimensions and shape the bodies resemble the uniseptate elongated conidia usually produced by *A. dactyloides,* while the swollen biseptate conidia occasionally produced by that species are not dissimilar to the ventricose spores typical of the present fungus. However, in *A. dactyloides* the conidia of the more exceptional type are borne on the same tall conidiophores as those of the usual type, and the two types show no marked difference either in the organization of their contents or in their readiness to germinate. Any presumed analogy with *A. dactyloides* is thus seriously disturbed; and it remains very uncertain whether the elongated bodies can be at all closely homologized with the ventricose conidia.

Of the several morphological features that might suggest a suitable epithet for the species, the bulbous apical modification of the conidiophore appears least apt to convey an objectionable connotation. At the risk, perhaps, of some exaggeration the fungus is described under a name compounded of two words meaning "pestle" and "form" respectively.

Dactylella doedycoides sp. nov.

Mycelium effusum; hyphis hyalinis, septatis, plerumque 2–4 μ crassis, laqueos circulares 20–36 μ latos in 3 cellulis arcuatis 16–28 μ longis medio 4–7 μ, extremo 2.5–5 μ crassis consistentes ex ramulo biloculari circa 10–20 μ longo 2.5–4 μ crasso proferentibus; his laqueis vermiculos nematodeos illaqueantibus, deinde tum per contractionem et inflationem trium cellularum animalia magnopere comprimentibus, ita haec trucidentibus, statim integumentum perforantibus, hyphas intus evolventibus quae carnem exhauriunt. Hyphae fertiles hyalinae, erectae, septatae, plerumque 225–500 μ altae, basi 5–8 μ crassae, sursum leviter attenuatae, prope apicem 2–3 μ crassae, apice abrupte tuberantes, ibi 3–5 μ crassae et unicum conidium ferentes. Conidia hyalina, turbinea, apice rotundata, deorsum paulum attenuata, basi abrupte truncata vel saepe aliquantulum cavata, 28–39 μ (saepe circa 34 μ) longa, 15–24 μ (saepe circa 20 μ) lata, vulgo biseptata—loculo infimo obconico, 4–10 μ (saepe circa 6.6 μ) longo; loculo medio dolioformi, ventricoso, 16–26 μ (saepe circa 22.7 μ) longo; loculo summo 2.5–7 μ (saepe circa 4.7 μ) longo.

Vermiculos nematodeos multarum specierum usque .6 mm. longos laqueans consumensque habitat in humo silvestri prope Butternut, Wisconsin.

Mycelium spreading; vegetative hyphae hyaline, septate, mostly

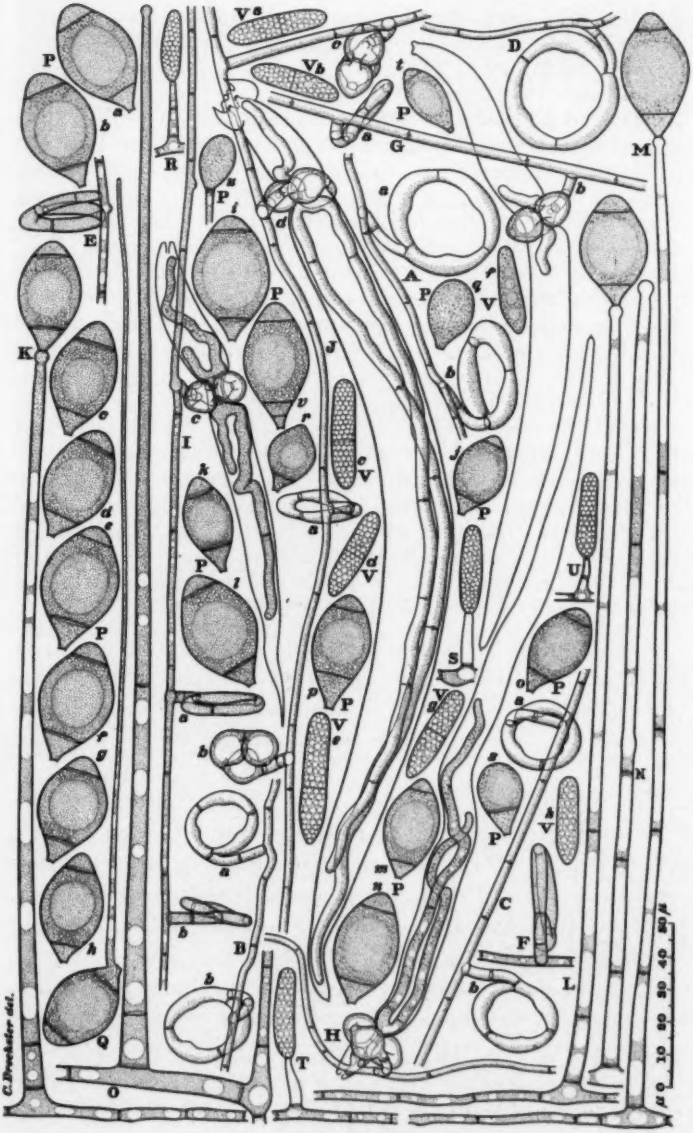

FIG. 1. *Dactylella doedycoides.*

2 to 4 μ wide, often, especially in the presence of nematodes, producing mostly underneath and at right angles to their axes approximately circular rings 20 to 36 μ in outside diameter, composed individually of 3 arcuate cells 16 to 28 μ long, 4 to 7 μ wide in the middle, and 2.5 to 5 μ wide at the ends—the first and third of the cells being united usually to each other as well as to the distal end of a somewhat curved or straight supporting branch 10 to 20 μ long, 2.5 to 4 μ wide, and consisting usually of 2 cells; following ensnarement of a nematode the individual ring through contraction and inflation of its component arcuate cells constricting the animal to death or into a state of reduced activity preceding death, then perforating the integument and extending lengthwise through the body assimilative hyphae that appropriate the fleshy contents. Conidiophores hyaline, erect, septate, 225 to 500 μ high, 5 to 8 μ wide at the base, tapering gradually upward to a diameter of 2 to 3 μ, then expanding abruptly into a knob-like tip 3 to 5 μ wide, whereon is borne a single conidium. Conidia hyaline, somewhat top-shaped, often tapering noticeably toward the abruptly or somewhat concavely truncate base, 28 to 39 μ (average 34 μ) long, 15 to 24 μ (average 20 μ) wide, usually divided by 2 septa into 3 cells—the basal obconical cell then measuring 4 to 10 μ (average 6.6 μ) in length, the middle swollen barrel-shaped cell measuring 16 to 26 μ (average 22.7 μ) in length, and the terminal cell measuring 2.5 to 7 μ (average 4.7 μ) in length.

Capturing and consuming nematodes measuring up to .6 mm. in length, referable to such genera as *Aphelenchoides, Plectus, Rhabditis, Diploscapter* and *Wilsonema,* it occurs abundantly in leaf mold in deciduous woods near Butternut, Wis.

A SPECIES OF DACTYLARIA WITH STALKED ADHESIVE KNOBS

Among several cultures of nematode-capturing fungi that Dr. M. B. Linford (**12**) isolated from Hawaiian soils and very kindly sent to me late in 1937 was a species of *Dactylaria* conspicuously different from any of the similarly predaceous hyphomycetous forms with which I had at the time become acquainted. Although the species had not been seen in the many cultures prepared, as occasion offered during the preceding four years, mainly with decaying vegetable refuse collected near Washington, D. C., it appeared in May, 1938, in a number of *Pythium* cultures to which had been added small pinches of somewhat woody, friable remnants from rotting stems of the giant ragweed, *Ambrosia trifida*

L., collected in Arlington, Va., a few weeks earlier. Addition of the ragweed detritus afforded development of bacteria in such meager quantity that nematodes addicted to ingestion of bacterial slime multiplied only sparingly, while forms equipped with a stylet and subsisting on protoplasm sucked from living *Pythium* filaments attained appreciably greater numbers. By far the most numerous of the eelworms expropriating the oomycetous mycelium was a species apparently identifiable as *Aphelenchoides parietinus* (Bastian 1865) Steiner 1932, whose frequent parasitism on fungus hyphae has been set forth by Christie and Arndt (2). Because of its abundance this species provided the chief source of nourishment for the new *Dactylaria*, which, wherever observed, was always found to have extended its mycelium from a deposit of ragweed refuse into the surrounding agar substratum. Other nematodes, including representatives of the genus *Rhabditis*, were also utilized by the fungus, suggesting that its development in the cultures was perhaps encouraged more by the relative freedom of the substratum from growth of putrefactive bacteria than by the taxonomic relationships of the eelworms available for consumption.

Although the new species of *Dactylaria* is correctly to be reckoned among the predaceous fungi, its attack on nematodes in my cultures was for the most part that of a parasite; for in most instances the animals were invaded by germ-tubes from adhering conidia. Usually several conidia were found attached to the anterior end of a nematode undergoing infection, so that as the encumbered animal continued to move about it gave somewhat the appearance of being provided with excessively long cephalic processes (FIG. 2, *A*). Infection was accomplished by narrow perforation of the integument, and extension into the animal of a germ tube from each conidium. The germ tubes continued their growth lengthwise through the nematode as branching hyphae, their advance being marked by fatty degeneration of musculature and internal organs. After appropriating the degenerating materials the internal hyphae would break through the integument and extend themselves into the surrounding substratum as new mycelial filaments on which would be borne at intervals stalked globose knobs (FIG. 2, *B–G*) generally similar to the predaceous knobs of *Dactylella ellipsospora* Grove and *D. asthenopaga* Drechsl. Much

as in these two species the globose knobs were functional in the capture of prey by means of a transparent adhesive substance secreted by them. A process arising from the individual knob would grow through the cushion of adhesive substance, narrowly perforate the animal's integument, and intrude a subspherical body that would disable the animal, thereby rendering it subject to invasion and expropriation by haustorial filaments.

With ample nourishment being supplied by captured animals, the fungus produces erect conidiophores that are shorter and more delicate than those of any other hyphomycetous form now known to prey on nematodes. Following development of a first conidium (FIG. 2, *H*) the axis of the conidiophore often elongates to produce a second one farther on, the first being pushed into a lateral position. Repetition of the process results in the development of several conidia in a loosely radiating head. The conidia are relatively uniform in structural design (FIG. 2, *N, a–z; O, a–z; P*), in nearly all instances being divided by four transverse septa into five cells. Four of these cells, approximately cylindrical in shape and nearly equal to one another in length, make up the main part of the spore. The fifth cell terminating the conidium is of a prolate ellipsoidal shape like the predaceous knobs borne on the mycelial hyphae. Though noticeably smaller than those knobs, it is nevertheless similarly adhesive, and functions in attaching the conidium to any active eelworm that may chance to come in contact with it. Occasionally a second adhesive knob may be found present at the proximal end of a detached spore (FIG. 2, *O, a, l, p*); its development possibly having taken place subsequent to disarticulation. Now and then a conidium may contain as many as eight cylindrical segments (FIG. 2, *O, k*), following division of some or all of the four cylindrical segments ordinarily present by median septa.

On removing its conidia from the conidiophores to sterile agar media by means of a small agar slab held on a flamed platinum spatula, the fungus may often be brought directly into pure culture, though at times further procedure may be necessary to remove contaminating bacteria. Even in the absence of extraneous organisms the mycelium is extended rather slowly on maizemeal agar, and when extended, is usually somewhat inconspicuous, owing

to meager development of aerial elements. Besides the hyphal anastomoses frequent in related species, the fungus shows in pure culture scattered knots composed of irregular hyphal branches intricated with one another. Sporulation is usually somewhat tardy, yet after several weeks numerous conidiophores are often present, many of them bearing individually 10 to 15 conidia in loose capitate arrangement (FIG. 2, *I, J*) on a sporiferous tip modified with slight geniculations and an occasional spur (FIG. 2, *K, L, M*). The conidia thus produced, like those found in nematode-infested cultures, are always provided distally with a globose adhesive knob.

In no other fungus so far known to prey on nematodes is the conidium consistently furnished with a special adhesive part at the time of its development. The nearest approach to such consistent modification is shown in *Dactylella leptospora* Drechsl., which often in pure culture and occasionally in nematode-infested cultures gives rise to conidia bearing terminally a globose knob. In *D. leptospora*, it is true, the conidial knobs have not been seen operative in attaching spores to active eelworms; whereas in the fungus under consideration the homologous modifications are conspicuously efficient in promoting a parasitic mode of attack. Presumably, however, this marked difference in observed usefulness derives from a difference in suitableness of some agar substrata for the operation of the conidial knobs produced by the two species, rather than from any essential difference in function.

A term compounded of two words meaning "to fasten" and "seed" respectively, is deemed an appropriate specific epithet for the fungus.

Dactylaria haptospora sp. nov.

Mycelium effusum; hyphis sterilibus septatis, hyalinis, 1.3–4.5 μ crassis, bullas globosas vel ellipsoideas 6–10 μ longas, 5–8.5 μ crassas, ex ramulo recto vel curvato, saepius 4–30 μ longo, circa 1.5–2.5 μ crasso, continuo vel 1–3-septato emittentibus; his bullis ad vermiculos nematodeos inhaerentibus, ita animalia tenentibus, integumentum eorum perforantibus, hyphas intrudentibus quae carnem exhauriunt. Hyphae fertiles hyalinae, erectae, septatae, 50–150 μ altae, basi 1.7–2.7 μ crassae, sursum leviter attenuatae, apice circa 1.5 μ crassae, ibi plus minusve geniculatae interdum parce ramosae, 1–15 conidia in capitulum laxum singulatim deinceps gerentes. Conidia hyalina, elongato-cylindrata, in toto 35–60 μ longa, medio 2.2–3.2 μ crassa, vulgo in 5 cellulis consistentia: cellula summa globosa vel ellipsoidea, 3–5 μ longa,

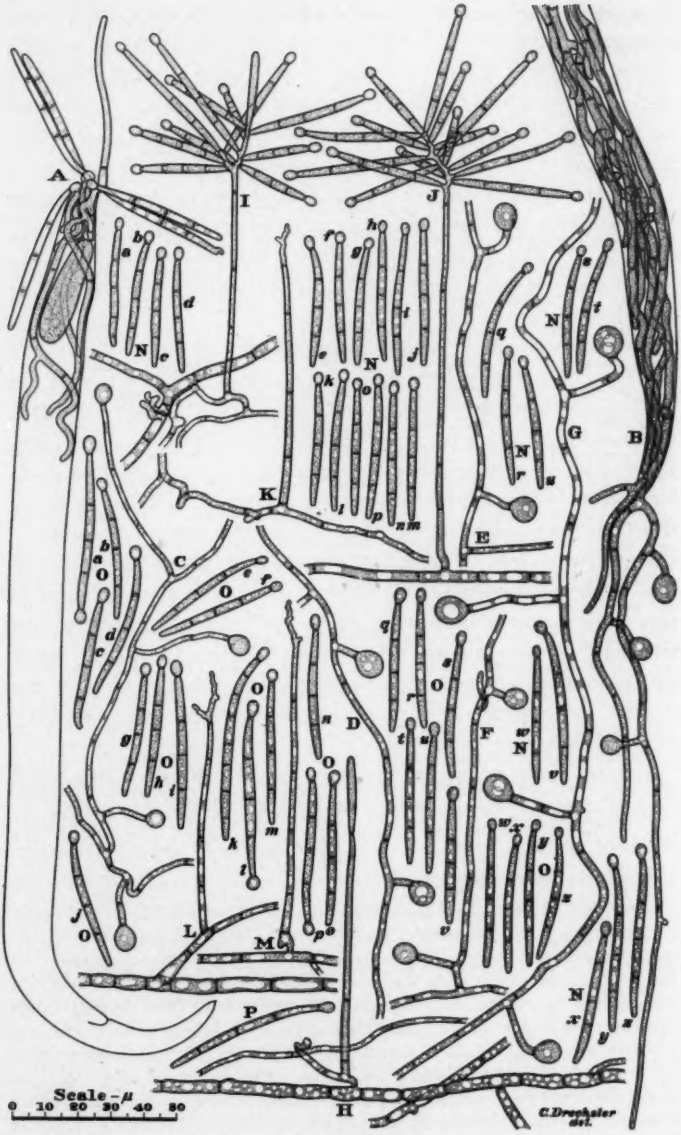

FIG. 2. *Dactylaria haptospora.*

2.5–3.7 μ crassa, glutinosa itaque saepe ad vermiculos nematodeos inhaerente; 4 alteris cellulis inter se subaequalibus, conjunctim partem cylindratam saepius sursum et deorsum plus minusve attenuatam conficientibus. Conidia rarius 5–8-septata, atque rarius non modo apice sed etiam basi cellula glutinosa instructa.

Vermiculos nematodeos diversos interficiens habitat in caulibus putrescentibus Ambrosiae trifidae in Arlington, Virginia, item in terra agresti in Hawaii.

Mycelium spreading; the vegetative hyphae hyaline, septate, 1.3 to 4.5 μ wide, often, especially in the presence of nematodes, giving rise here and there on stalks frequently straight or somewhat curved, 4 to 30 μ long, 1.5 to 2.5 μ wide, continuous or containing 1 to 3 septa, to unicellular adhesive knobs subspherical or prolate ellipsoidal in shape, 6 to 10 μ long and 5 to 8.5 μ wide; the knobs holding fast to nematodes, individually perforating the integument of the adhering animal, and intruding haustorial filaments lengthwise through its body to appropriate the fleshy contents. Conidiophores hyaline, erect, septate, 50 to 150 μ high, 1.7 to 2.7 μ wide at the base, very gradually tapering upward to a width of about 1.5 μ, then terminating often in a slightly branched more or less geniculate sporiferous part formed by repeated elongation following successive apical production of conidia up to 15 in number in loosely capitate arrangement. Conidia hyaline, elongated cylindrical, 35 to 60 μ long, 2.2 to 3.2 μ wide in the middle, usually divided by 4 septa into 5 cells: the distal cell globose or prolate ellipsoidal, 3 to 5 μ long, 2.5 to 3.7 μ wide, often adhering to a nematode and directly operative in causing its infection; the other 4 cells approximately equal to one another in length, together making up an elongated part often tapering more or less both toward its base and toward the tip. Conidia more rarely containing 5 to 8 cross-walls, and more rarely, too, provided with an adhesive globose cell not only at the apical end but also at the basal end.

Destroying various species of nematodes it occurs in decaying woody remnants of *Ambrosia trifida* in Arlington, Va., and also in field soils in Hawaii.

A SPECIES OF TRIDENTARIA PREYING ON WEAKENED EELWORMS

A fungus of unusual predaceous behavior made its appearance in a maizemeal-agar plate culture originally used in isolating a species of *Pythium* from some discolored roots of a smartweed (*Persicaria Hydropiper* L.) plant dug up in a moist field near

Butternut, Wis., early in September, 1938. When the culture was several days old and completely permeated with *Pythium* mycelium, it had been further planted with pinches of leaf mold taken from deciduous woods near Butternut, Wis., early in September, 1938. Thirty days after this final addition of vegetable material a tract of the substratum embracing about 100 square millimeters and adjacent to one of the leaf-mold deposits was found sparsely permeated by a delicate septate mycelium reminiscent of the sparse mycelium of *Triposporina aphanopaga* Drechsl. The fungus had evidently grown out from the forest refuse, and seemed to be wholly dependent for nourishment on the nematodes captured by it.

Destruction of nematodes by the fungus was on a relatively small scale; the number of animals consumed altogether probably not exceeding five hundred. All of the eelworms examined soon after their capture, before their morphological features had become obliterated beyond recognition, appeared to belong to a single species tentatively identified as *Aphelenchoides parietinus*. The captives, whether large or small, invariably showed rather extensive infection, especially in their median and posterior parts, by an organism producing aggregations of subspherical spore-like bodies about 1.5 μ in diameter, each containing a somewhat eccentrically placed refractive globule approximately .6 μ in diameter. Continued accumulation of these minute bodies, which in shape and structure appeared closely comparable to some of the spores observed in nematodes by Micoletzky (**13**: p. 285–286, Taf. XI, 49 c; 50 c, d), was accompanied by progressive enfeeblement and finally complete disablement of the animal. Only nematodes already much enfeebled by the internal parasite were taken by the fungus.

This limitation in predaceous activity has its cause apparently in the meager differentiation of the mycelial elements operative in effecting capture. Repeated examination failed to disclose any evidence that vegetative filaments ever give rise to structures recognizable as predaceous apparatus previous to the moment prey is engaged and held; though, to be sure, captured nematodes are usually found encircled by a hyphal branch (FIG. 3, *A–D*) in a manner recalling the encirclement of eelworms by non-constricting rings of *Dactylella leptospora* and *Dactylaria candida* (Nees)

Sacc. In some instances the encircling element may even constitute a ring with a closure of contact if not of anastomosis (FIG. 3, *A*). More frequently, however, the tip of the element is definitely free, and the impression given is that encirclement came about through growth of the branch around the animal after its capture (FIG. 3, *B; C; D, c, d*). Unless appearances are misleading the animal is held at the beginning merely by adhesion to the distal part of a hyphal branch. If the branch is not already wider than the parent mycelial filament, it undergoes some widening subsequently, either throughout its length, or at times more especially in its distal portion. As the thickened branch continues its growth somewhat laterally in giving rise to the encircling element, its adhering tip narrowly perforates the integument of the captive and sends haustorial hyphae lengthwise through the fleshy interior. Some nematodes (FIG. 3, *D, b*) are captured and encircled by two separate branches (FIG. 3, *D, c, d*); and, again, a branch (FIG. 3, *D, c*) after participating in the capture of one eelworm (FIG. 1, *D, b*) may continue growth and capture a second (FIG. 1, *D, a*). Development of haustorial filaments is very often noticeably less abundant than in nematodes preyed upon by other hyphomycetes—the more meager development having an obvious explanation in the partial appropriation of available materials by the minute parasite.

Conidiophores were found arising here and there from the tract of mycelium (FIG. 3, *E–J*). In stature they show closest similarity to the fertile hyphae of *Dactylella tenuis* Drechsl. The conidia (FIG. 3, *K, a–z, L*) produced on them singly are of unusually distinctive make-up, being composed as a rule of a short obconical two-celled basal part together with three digitate or prong-like distal parts, each of which is divided by three to five transverse septa into four to six cells. In number of conidial prongs the fungus at once recalls *Tridentaria carnivora* Drechsl., a member of the predaceous series that was described in an earlier paper (**9**) as subsisting by the capture of the testaceous rhizopod *Difflugia constricta* (Ehrenb.) Leidy. However the relationship of the prongs to the basal part is different in the two species; for in *T. carnivora* the basal part is prolonged directly into one of the prongs, whereas in the present species it bears all three prongs

alike as terminal branches in isogonal arrangement. Accordingly, while the conidia typical of *T. carnivora* are symmetrical with reference to only one plane, those of the present species are symmetrical with reference to three planes.

The isogonal branching evident in conidia of the fungus under consideration is more closely approximated in the tridental conidia (**7**: p. 396, fig. 1, *Q, R*) occasionally produced by *Pedilospora dactylopaga* Drechsl., a hyphomycetous form which I first described as predaceous only on the testaceous soil rhizopods *Difflugia globulosa* Duj. and *Trinema enchelys* Ehrenb., but which I have since found preying also on *Sphenoderis dentata* Pen. (**10**: p. 405–406) and *Geococcus vulgaris* Francé. Similarity in angular relationships is likewise apparent when the symmetrically two-pronged conidia typical of *P. dactylopaga* are compared with the two-pronged conidia (FIG. 3, *K, f, z*) produced occasionally by the present fungus. The symmetrical dichotomy in the bidentate conidia of both these forms is readily distinguished from the monopodial branching evident in the bidentate conidia produced now and then by *Tridentaria carnivora* (**9**: p. 392, fig. 1, *I*).

Germination of the curiously designed asexual spores takes place by the production of germ-tubes from the basal end and from the tips of the prongs (FIG. 3, *M*). As in other members of the predaceous series of hyphomycetes, anastomoses of fallen conidia with mycelial hyphae occur frequently (FIG. 3, *N*).

The fungus is referred to *Tridentaria* Preuss, a genus so poorly defined by its author (**14**) that misgivings as to the wisdom of retaining it have been expressed (**11**). When earlier I nevertheless ventured to commit *T. carnivora* to this little esteemed taxonomic repository, I was persuaded as much by the realistic appropriateness of the generic term invented by Preuss as by the sketchy definition presumably intended to govern its application. The term appears, if anything, even more felicitous in relation to the conidium of the present species, especially when this structure is considered in combination with the supporting conidiophore. A word meaning "encircling," which it is hoped may prove conveniently suggestive of the unusual predaceous behavior of the fungus, is proposed as a suitable specific name.

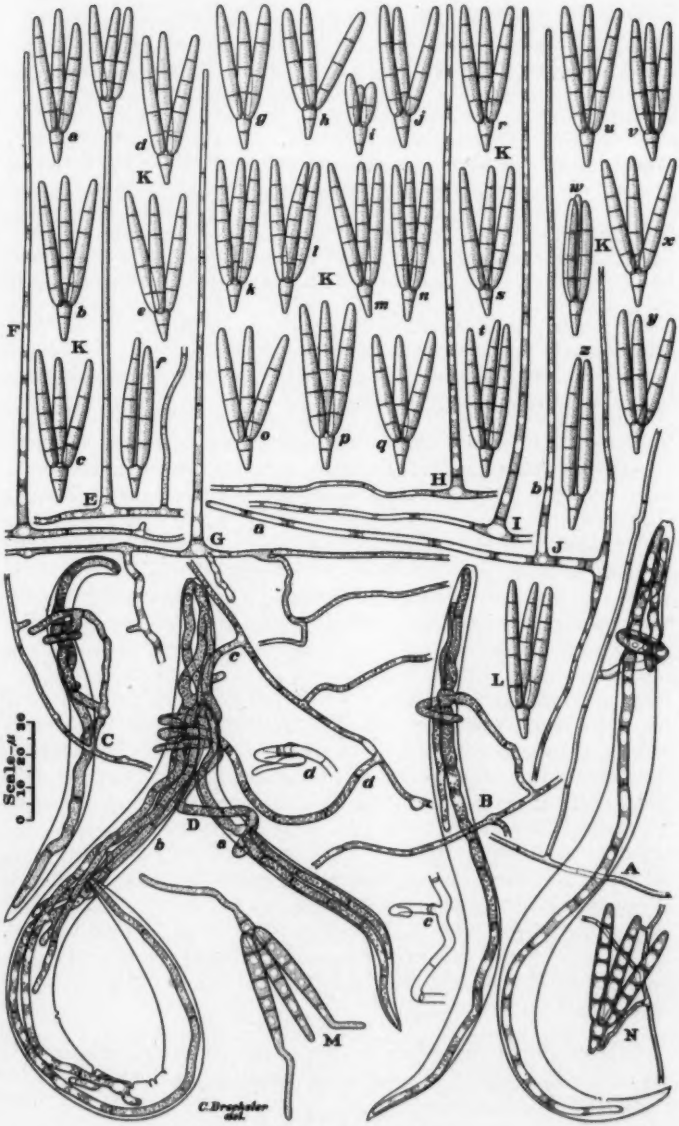

FIG. 3. *Tridentaria implicans.*

Tridentaria implicans sp. nov.

Mycelium sparsum; hyphis sterilibus hyalinis, parce ramosis, mediocriter septatis, vulgo 1–2.5 μ crassis, ramos saepius 15–75 μ longos, 2–3.5 μ crassos gerentibus—his crassioribus ramis vermiculos nematodeos attingentibus et haerendo tenentibus, animalia implicantibus, integumentum eorum perforantibus, hyphas usque 4 μ crassas intrudentibus quae carnem exhauriunt. Hyphae fertiles erectae, hyalinae, septatae, simplices, 100–200 μ altae, basi circa 3.5 μ crassae, sursum leviter attenuatae, apice circa 1.5 μ crassae, unicum conidium ferentes. Conidia hyalina, raro bifurca in modum conidiorum generis *Pedilosporae*, vulgo ex 4 partibus ad instar fuscinae composita—parte infera quae hastile fuscinae facit obconica, uniseptata, basi 1–1.5 μ crassa, sursum latescente, apice rotundata, 4–5.5 μ crassa, ibi 3 dentes aliquantulum divaricatos ferente; dentibus digitiformibus, 12–42 μ saepissime 30–40 μ longis, 3.5–5 μ crassis, plerumque 3–5-septatis, basi rotundatis, sursum nonnihil attenuatis.

Vermiculos nematodeos debilitatos capiens consumensque habitat in humo silvestri prope Butternut, Wisconsin.

Mycelium in mixed culture on agar substrata rather meager; vegetative hyphae hyaline, sparingly branched, septate at moderate intervals, commonly 1 to 2.5 μ wide, giving rise here and there to slightly differentiated branches often 15 to 75 μ long and 2 to 3.5 μ wide; these branches capturing nematodes by adhesion, then individually continuing growth to encircle the captive in 1 to 1.5 turns while narrowly perforating its integument and extending lengthwise through its interior assimilative hyphae up to 4 μ wide that appropriate the fleshy contents. Conidiophores erect, hyaline, septate, simple, 100 to 200 μ high, usually about 3.5 μ wide at the base, tapering gradually upward to a width of 1.5 μ at the tip, and there bearing a single conidium. Conidia hyaline, now and then bifurcate in the manner typical for conidia of the genus *Pedilospora*, but usually composed of 4 parts in trident-like arrangement—the basal part corresponding to the shaft of a trident being obconical, transversely uniseptate, 1 to 1.5 μ wide at the base, widening upward to a diameter of 4 to 5.5 μ at the bluntly rounded apex whereon are borne in isogonal arrangement the other 3 slightly divergent digitate parts corresponding to prongs of a trident; the prong-like parts 12 to 42 μ (usually 30 to 40 μ) long, 3.5 to 5 μ wide, individually tapering noticeably upward from a broadly rounded basal end, and divided by 3 to 5 transverse septa into 4 to 6 cells.

Capturing and subsisting on weakened nematodes it occurs in leaf mold of deciduous woods near Butternut, Wis.

CORRECTIONS CONCERNING DACTYLARIA BROCHOPAGA AND ARTHROBOTRYS SUPERBA CORDA

An incidental comment in a recent review (**1**: lines 20, 21) calls attention to an error of more than ordinary seriousness in my earlier paper on nematode-capturing hyphomycetes. The species which I intended to name *Dactylaria brochopaga,* and to which I referred in all other connections by that binomial, was, owing to a lapse of the pen, presented in its formal diagnosis under the binomial *Dactylella brochopaga* (**8**: p. 517, line 7). Despite the unhappy *lapsus calami* the species has fortunately been cited under its correct generic name by Comandon and de Fonbrune (**3, 4**), as well as by Roubaud and Deschiens (**15**). It is hoped that the binomial *Dactylaria brochopaga* will continue to be used in all future references to the fungus.

Rather curiously the somewhat troublesome similarity in spelling between the generic names *Dactylaria* and *Dactylella* is associated with an equally troublesome morphological intergradation between those members of the two genera that constitute members of the predaceous series. When developing on natural or on nematode-infested agar media, the fungi in question offer little difficulty, since on these materials their conidiophores ordinarily bear either a solitary terminal conidium or a terminal cluster of conidia. The generic difference tends to become more or less obliterated when some of the fungi are grown in pure culture on agar media; for a number of capitate forms, including *Dactylaria brochopaga,* then often produce their plural conidia in extended arrangement, and certain forms more usually seen with solitary conidia, as, for example, *Dactylella spermatophaga* Drechsl., then likewise often produce plural conidia in extended arrangement on a conidiophore successively prolonged. On the other hand, two capitate forms, namely *Dactylaria thaumasia* and *Dactylaria haptospora* Drechsl., often appear somewhat more pronouncedly capitate when grown in pure culture, owing to a more copious production of conidia.

In my earlier paper, too, similarity of spelling facilitated an error (**8**: p. 454, line 28) resulting in a rather obvious misstatement concerning the conidia of *Arthrobotrys superba.* This mis-

statement is to be remedied by substituting for the unintended "uniseptate" the correct word "unseptate."

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EXPLANATION OF FIGURES

FIG. 1. *Dactylella doedycoides;* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A, B, C,* Portions of mycelial hyphae, each bearing two open constricting rings, *a* and *b,* that have been jostled by passing nematodes from their original approximately vertical positions into more nearly horizontal positions where their cellular make-up is better revealed. *D,* Portion of a mycelial hypha bearing an unusually large open predaceous ring that has been jostled into a nearly horizontal position. *E, F,* Portions of mycelial hyphae, each bearing an open predaceous ring apparently in its original position. *G,* Portion of mycelial hypha bearing two predaceous rings, one of them, *a,* being shown open, the other, *b,* having strangled a small nematode possibly identifiable as a larva of *Aphelenchoides parietinus,* and intruded into the captive two short haustorial branches. *H,* Portion of mycelial hypha bearing a predaceous ring that has strangled a nematode possibly referable to *Plectus parvus* Bastian, and extended two haustorial filaments through half the length of the animal. *I,* Portion of mycelial hypha bearing two open predaceous rings, *a, b,* apparently in their original positions, and a third ring, *c,* that after capturing and strangling a small specimen of *Rhabditis dolichura* Schneider has invaded the captive by means of four haustorial filaments. *J,* Portion of mycelium bearing one open predaceous ring, *a,* two predaceous rings, *b, c,* that have closed without capturing prey, and a fourth ring, *d,* that after strangling an eelworm probably referable to *Wilsonema* sp., has extended haustorial hyphae throughout the length of the captive. *K, L, M,* Portions of hypha, each with an erect conidiophore whereon is borne a mature conidium. *N,* Conidiophore denuded of the conidium it produced. *O,* A denuded conidiophore that was produced from the basal portion of an older one, which now is in contact with the substratum. *P,* Conidia: *a–p,* mature specimens of usual type with two septa and more or less concave basal end; *q,* non-septate specimen that probably became detached while still immature; *r, s,* small specimens, apparently mature, though containing only one septum; *t,* small specimen with two septa, the proximal being so close to the convex basal end that it may possibly correspond to the usual basal membrane; *u,* young specimen set off from the sporophore by two septa very close together, the upper one possibly corresponding to the usual basal membrane, the lower one possibly corresponding to the basal membrane in exceptional specimens such as *t; v,* large mature specimen with septation unusual for the species, the three cross-walls showing the positional arrangement most frequent in conidia of *Dactylella bembicodes* and *Dactylaria thaumasia. Q,* Conidium germinating by production of a delicate erect germ sporangiophore. *R–U,* Short erect sporophores, each bearing an elongate spore that shows globulose internal structure and apparently is not homologous to a conidium. *V, a–h,* Elongate spores detached from erect sporophores, all of them except the apparently immature specimen *f,* showing characteristic globulose internal structure.

FIG. 2. *Dactylaria haptospora;* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* Specimen of *Aphelenchoides parietinus* invaded at its anterior end by infection hyphae coming from five conidia adhering to the head; one of the conidia having apparently

germinated before becoming attached to the animal. (The oblong body shown in the nematode is a young thallus of the phycomycetous parasite *Haptoglossa heterospora* Drechsl.) *B*, Portion of a specimen of *A. parietinus* permeated with mycelium of the fungus; from the tail-end of the animal have grown out a few vegetative hyphae whereon are borne three stalked adhesive knobs. *C*, Portion of mycelium with four adhesive knobs. *D*, *E*, *F*, Portions of mycelium, each bearing two stalked predaceous knobs. *G*, Portion of hypha with four stalked predaceous knobs. *H*, Portion of mycelium with an erect conidiophore, which terminates in an immature conidium, the first to be produced by it. *I*, Portion of mycelium with an erect conidiophore on which are borne 11 mature conidia, a twelfth conidium being in process of development. *J*, Portion of hypha from which has arisen an erect conidiophore bearing 15 mature conidia. *K*, *L*, *M*, Portions of mycelium, each bearing a denuded conidiophore. *N*, *a–z*, *O*, *a–z*, *P*, Conidia showing variations in shape, dimensions, septation and number of adhesive organs.

FIG. 3. *Tridentaria implicans;* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, *B*, *C*, Nematodes, possibly referable to *Aphelenchoides parietinus*, that after being weakened through extensive infection by a minute internal parasite, were captured individually by adhesion to a branch of the fungus, entwined by a hyphal prolongation, and invaded by haustorial filaments. *D*, Portion of mycelium with two captured nematodes *a*, *b*—nematode *a* having been captured, enveloped, and invaded by the distal part of branch *c;* while nematode *b* was captured, enveloped, and invaded conjointly by the branches *c* and *d;* the portions of branches *c* and *d* enveloping nematode *b* being also shown separately for clearness. *E*, Portion of hypha with an erect conidiophore on which a conidium is borne. *F–I*, Portions of mycelium, each with an erect denuded conidiophore. *J*, Portion of a hypha on which was produced a conidiophore, *a*, that after serving its primary function, declined to the substratum and gave rise to a secondary conidiophore. *K*, *a–z*, *L*, Conidia showing variations in number and dimensions of shaft and prongs, and in abundance of septa. *M*, Conidium germinating by production of germ tubes from base of shaft and from tips of two prongs. *N*, Conidium anastomosing with branches of a vegetative hypha.

NUCLEAR MIGRATION IN GELASINOSPORA

ELEANOR SILVER DOWDING AND A. H. REGINALD BULLER

(WITH 6 FIGURES)

I. INTRODUCTION

In 1931, the senior author (Buller) discovered that, in *Coprinus lagopus*, during the initiation of the sexual process, nuclei migrate from one mycelium into another (**1**). Using two haploid mycelia of opposite sex, (*AB*) and (*ab*), he showed that a large mat of the mycelium (*AB*) could be diploidised by a small inoculum of mycelium (*ab*), so that all the growing hyphae of (*AB*) produced clamp-connexions (indicating the presence of conjugate pairs of nuclei); and he also showed that the rate of migration of the (*ab*) nuclei through the mycelium (*AB*) was about ten times the rate of elongation of the hyphae.

In 1933, Craigie (**4**) showed that, in the Uredinales, the union of a pycnidiospore with a flexuous hypha of opposite sex leads to the diploidisation of the haploid proto-aecidia, with the result that the proto-aecidia develop into aecidia. It is obvious that, after a pycnidiospore has united with a flexuous hypha of opposite sex, the nucleus of the pycnidiospore must migrate down the flexuous hypha, through the wall of the pycnidium, and through the vegetative intercellular mycelium in the leaf to the proto-aecidia. That nuclear migration through a mycelium takes place in the Uredinales may therefore be regarded as having been established.

In 1935, nuclear migration in the Ascomycetes was demonstrated by Dodge (**5**). He placed two mycelia of *Neurospora tetrasperma*, S3 and S9, which were of opposite sex, on opposite sides of an agar plate. Perithecia began to form as usual about the fourth day on the S9 mycelium on one side of the line of meeting. Four small blocks of agar bearing a very few young perithecia were then transferred to separate plates which were incubated for twelve hours. The hyphae of the mycelium in the blocks grew out so that single hyphal tips could be isolated very easily. Seven 1-tip

tube cultures were obtained from each of the four transplants. Later they all produced perithecia, which showed that the single tips isolated must have carried both kinds of nuclei. Said Dodge: "Since mycelia S3 and S9 with which the plate was inoculated are both unisexual, the bisexual tips isolated later from the S9 side must have represented new branches that grew out from the old S9 mycelium after it had received the S3 nuclei passed along down following anastomoses at the line of meeting." Dodge made similar experiments with *Gelasinospora tetrasperma.*

Some new experiments on nuclear migration in *Gelasinospora tetrasperma* will now be described. They were designed with a view to demonstrating once again the fact of nuclear migration in a multicellular mycelium and to solving problems concerned with: (1) the speed of nuclear migration; (2) the effect of light on nuclear migration; (3) the path of nuclear migration from cell to cell; and (4) the relation of nuclear migration to cytoplasmic streaming.[1]

II. MATERIAL AND METHODS

The experiments were made with *Gelasinospora tetrasperma.* This species, one of the Pyrenomycetes, was described by Dowding in 1933 (**6**). It resembles *Neurospora tetrasperma* in that its asci contain normally four bisexual spores and occasionally, in addition to bisexual spores, one or two dwarf unisexual spores. Some of these dwarf spores were isolated and sown on nutrient agar. In this way haploid mycelia were obtained.

Gelasinospora tetrasperma grows vigorously in culture and is devoid of secondary spores. The absence of secondary spores eliminates a possible source of contamination.

The fungus is known to include two races, a Canadian race and an English race, which are sexually incompatible with one another (**6**). Both these races were used, but most of the experiments were made with two haploid mycelia of the Canadian race. These mycelia will be referred to as *mycelium no. 3* and *mycelium no. 4.* When mated they were found to be of opposite sex.

Difco malt-agar was used as a culture medium. To obtain the

[1] An abstract of this paper was read at the Third International Congress for Microbiology, in New York, on September 7, 1939.

best experimental results it was found necessary to work with mycelia recently developed from spores and to place the cultures under favourable conditions of light and oxygen.

Age of mycelium. Unisexual mycelia that had been grown from dwarf spores and then stored in the refrigerator for five years were found to be unsuitable as material for the investigation because, when two of the mycelia of opposite sex were mated, they showed but limited nuclear migration.

For the critical experiments four unisexual mycelia obtained freshly from as many dwarf spores were employed.

Light. Both in darkness and in light rudiments of perithecia develop on bisexual mycelia in about three days; but the development of these rudiments into perithecia is strongly influenced by light.

Bisexual mycelia, when well illuminated, develop perithecia within five days; whereas, when kept in the dark, they produce perithecia only after the lapse of two weeks.

In the experiments here described, unless otherwise stated, the cultures were grown either in direct sunlight or about two feet from a 100-watt electric-light bulb which was kept running night and day. A shallow glass of water between the culture and the source of light prevented the agar from being dried by the heat of the lamp.

Oxygen. At first the Petri dishes and the test-tubes were sealed with strips of "parafilm" as a precaution against contamination; but this technique was abandoned when it was observed that perithecia do not develop in a vessel that is tightly sealed. Even without the parafilm, Petri-dish cultures grown in humid air may become sealed from within by an excessive growth of aerial mycelium, and then they do not fruit. On account of the adverse effect of sealing, the cultures were usually grown in Petri dishes without lids. The dishes were set on a glass plate and covered with a large glass vessel, and the vessel contained damp blotting paper placed in such a way as to reflect the light on to the cultures.

Nuclei. Material for studying the nuclei was fixed in Duggar's Gilson Fluid containing a few crystals of urea and then stained in 0.05 per cent Heidenhain's haematoxylin. The cytological work

was carried out while one of us (Dowding) was a guest of the Department of Botany at the University of Michigan.

III. DEMONSTRATION OF NUCLEAR MIGRATION

Convincing evidence that nuclei migrate from one mycelium into another was obtained as follows.

A large Petri dish, 15 cm. in diameter, was filled with agar. Mycelium no. 3 and mycelium no. 4, which were of opposite sex, were placed on opposite sides of the dish, and the agar in the centre was cut away so that the bottom of the dish became exposed

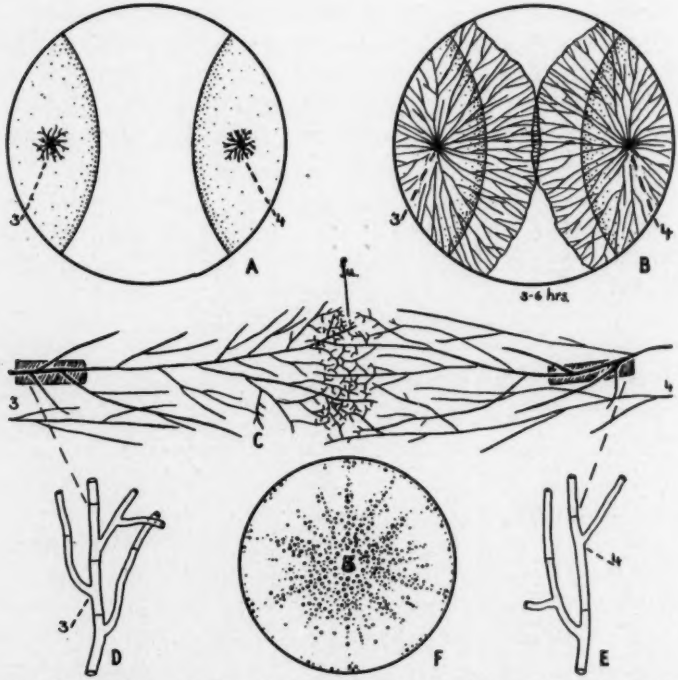

FIG. 1. Nuclear migration in *Gelasinospora tetrasperma*. Explanation in the text.

(FIG. 1, *A*). The two mycelia grew out past the agar and over the glass until they met (FIG. 1, *B*). They grew past each other

for a distance of about 2 mm., branched into a fine network, and fused with one another (FIG. 1, *C*). From three to six hours after fusion had been observed, a little sterile water was poured over the plate so that the hyphae would not adhere to the glass and then, with the aid of a binocular dissecting microscope, an area was chosen on the mycelium no. 3 side, about 5 mm. from the fusion zone, in which each hypha could be clearly seen to be part of mycelium no. 3. In this area a length of two or three cells of a main hypha was cut out and transferred to fresh agar (FIG. 1, *C* and *D*). The same procedure was carried out with a portion of the mycelium no. 4 (FIG. 1, *C* and *E*). In less than a week, the two cultures derived from the hyphal transplants of mycelium no. 3 and mycelium no. 4 had both produced perithecia. The perithecia on the transplant of mycelium no. 3 are shown in figure 1, *F*.

From the observations just described we may conclude that, in the mycelia shown in figure 1, *C*, nuclei had passed from mycelium no. 4 into mycelium no. 3 and from mycelium no. 3 into mycelium no. 4.

The experiment was repeated with this difference: that the time permitted to elapse after the two mycelia had fused and before hyphal transplants were made was lengthened to 24 hours in some plates and to 48 hours in other plates. It was then found that not one of the thirty-three transplants that were set on fresh agar produced perithecia. It would appear that within the 24 or 48 hours the migrating nuclei had migrated through and past the cells that had been transplanted.

IV. POSSIBILITY OF THE OVERGROWTH OF TWO MYCELIA

In *Gelasinospora tetrasperma*, when the mycelia nos. 3 and 4 are paired on an agar plate, the perithecia are frequently limited to the mycelium no. 4 (FIG. 4, *A*). In view of the results obtained in the experiment described in Section III, this distribution of perithecia is probably due to the migration of nuclei of mycelium no. 3 into mycelium no. 4. The alternative explanation is that the mycelium no. 3 overgrows mycelium no. 4, as suggested by Colson for *Neurospora tetrasperma* (**3**, p. 217). The experiment now to be described shows that, in *Gelasinospora tetrasperma* under cultural conditions, one mycelium does not overgrow another.

As material for the experiment both the Canadian race and the English race were employed. These races are sexually incompatible and, when paired, do not give rise to hybrid perithecia (6).

A Canadian unisexual mycelium and an English bisexual mycelium were paired on a plate. After seven days, the two mycelia had grown out, so that each occupied one-half of the plate, and perithecia had appeared on the bisexual mycelium (FIG. 2, *A*). A block of fresh agar was then placed on the top of the unisexual mycelium (FIG. 2, *A*); but, even after a month, no perithecia appeared on the block of agar or on any part of the half of the dish occupied by the unisexual mycelium (FIG. 2, *A*).

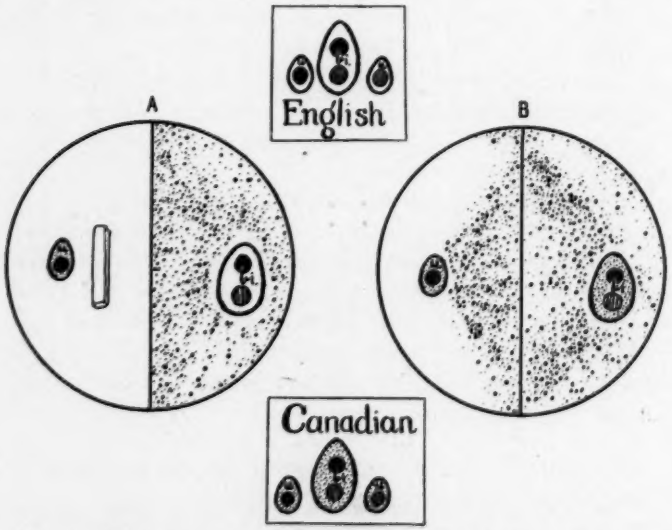

FIG. 2. *Gelasinospora tetrasperma.* One mycelium does not overgrow another. Explanation in the text.

It thus appears that, in the experiment, the bisexual mycelium remained limited to that half of the dish in which it was planted and did not overgrow or intermingle with the unisexual mycelium.

A Canadian unisexual mycelium and a Canadian bisexual mycelium were paired on a plate. In this experiment, unlike the one just described, perithecia appeared on both sides of the plate (FIG.

2, *B*). Here the two mycelia, both belonging to the Canadian race, were sexually compatible, and nuclei of the required sex migrated from the bisexual into the unisexual mycelium.

The two experiments just described and illustrated in figure 2 were repeated several times and always with the same result.

V. SPEED OF NUCLEAR MIGRATION

To measure the speed of nuclear migration and to compare it with the hyphal growth-rate, two sets of experiments, *A* and *B*, were made. An account of these experiments will now be given.

A. Paired mycelia. Inocula of the two unisexual mycelia, nos. 3 and 4, were placed on opposite sides of an agar plate and were allowed to grow until they met (FIG. 3, *A*). A millimeter scale was then drawn with ink on the bottom of the plate in such a way that the zero mark was on the meeting line of the two mycelia (FIG. 3, *A*).

Five hours after the two mycelia had met, six transfers were made from the plate to tubes of fresh agar. Three of the transfers were taken from mycelium no. 3 and three from mycelium no. 4; and, on each side of the median meeting line, they were taken at distances of 15 mm., 20 mm., and 25 mm. (FIG. 3, *A*). Within a week two of the six transfers had produced perithecia, while the other four remained sterile. The transplants for the two tube cultures that fruited were taken as follows: one 15 mm. and the other 20 mm. from the meeting line on the mycelium no. 4 side of the dish.

On repeating the experiment just described, once more two of the six transfers fruited and again one of the transfers had been taken 15 mm. and the other 20 mm. from the meeting line of the two mycelia. However, in this experiment, the mycelium that yielded the fruiting transfers was mycelium no. 3 and not mycelium no. 4.

We may conclude: (1) that, in the first of the two experiments just described the nuclei of mycelium no. 3 travelled into mycelium no. 4; (2) that, in the second experiment, the nuclei of mycelium no. 4 travelled into the mycelium no. 3; (3) that, in both experiments, the migrating nuclei travelled a distance of 20 to 25 mm.

in five hours; and (4) that the velocity of the migrating nuclei was 4 to 5 mm. per hour.

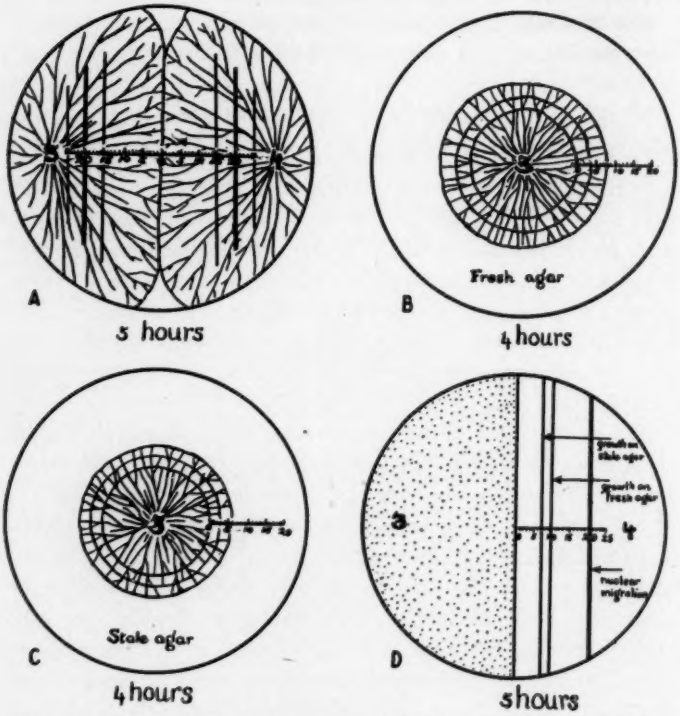

FIG. 3. *Gelasinospora tetrasperma.* Speed of nuclear migration, and a comparison of this speed with the growth-rate of the hyphae. Explanation in the text.

Two more plates were inoculated with mycelium no. 3. One contained fresh malt-agar (FIG. 3, *B*) and the other staled malt-agar (FIG. 3, *C*). The staled agar was obtained by melting the agar of a two-day-old culture of mycelium no. 4 by heating it over warm water and then pouring the melted agar on to a fresh plate and discarding the mycelium. After the inoculum of mycelium no. 3 had grown out a short distance into the agar in the two plates, the increment of growth was recorded every two hours by

ringing the edge of the mycelium on the under side of the dish with ink (FIG. 3, *B* and *C*). The radial growth rate of mycelium no. 3 was found to be: on fresh agar, 2 mm. per hour; and on agar staled by mycelium no. 4, 1.5 mm. per hour.

Figure 3, *D*, shows diagrammatically the results of the experiments illustrated in figure 3, *A*, *B*, and *C*. In a period of five hours, mycelium no. 3 grew on fresh agar 10 mm. and on stale agar 7 mm. and the nuclei of mycelium no. 3 migrated into the mycelium no. 4 a distance of at least 20 mm. From these data we may conclude that the rate of movement of the nuclei was twice the rate of hyphal growth on fresh agar and nearly three times the rate at which the hyphae would have grown had one mycelium overgrown the other and thus had been forced to make its way through stale agar.

B. Patched mycelia. Two mycelia of *Gelasinospora tetrasperma* of opposite sex were grown for two days on two agar plates. One of the two mycelia was then used to *patch* the other: from one dish a small rectangular piece of hyphae-containing agar (10 × 5 mm.) was cut out, and it was set upside down on the mycelium in the other dish along a radius of the dish and about half way between the centre of the dish and the periphery. In about a week, perithecia formed on the agar surrounding the patch and as far away from the patch as 15 mm. (FIG. 4, *D*). Patched mycelia were employed in a second method devised for measuring the speed of nuclear migration. An experiment made with patched mycelia will now be described.

Three patches of mycelium no. 3 were applied to a plate containing mycelium no. 4. Ten hours later, three transfers were removed from the agar surrounding each patch at distances of 5 mm., 10 mm., and 15 mm. from the edge of the patch. The transfers removed at distances of 5 mm. and 10 mm. from the patches produced perithecia, but those removed at a distance of 15 mm. remained sterile.

In the experiment just described the nuclei derived from the patch of mycelium no. 3 must have travelled through the hyphae of mycelium no. 4 a distance of 10 mm. in 10 hours, or at a speed of one mm. per hour. This speed is much less than the speed of from 4 to 5 mm. per hour obtained in the experiments made with

paired mycelia. The relatively low speed of nuclear migration in the patched mycelium may well have been due to the fact that, in cutting out the patches, the hyphae along the edges of each patch were severed by the knife and thus injured. However, a speed of one mm. per hour exceeds the rate of elongation of the hyphae, as will now be shown.

Two patches of mycelium no. 3 were applied to two agar plates of which one contained fresh malt-agar and the other stale agar obtained from a culture in which mycelium no. 4 had been growing for two days. Ten hours after the plates had been inoculated, the hyphae in the patch on the fresh agar had grown out 7 mm. while the hyphae in the patch on the stale agar had grown out 6 mm. It is obvious that these growth rates, 0.7 mm. per hour and 0.6 mm. per hour respectively, are much less than the rate of nuclear migration, namely, 1.0 mm. per hour, obtained in the preceding experiment.

VI. THE EFFECT OF LIGHT ON NUCLEAR MIGRATION

As has already been remarked, when mycelia nos. 3 and 4 are paired, the perithecia frequently are limited in their distribution to mycelium no. 4 (FIG. 4, *A*). Sometimes, however, the reverse is true and the perithecia are limited to mycelium no. 3.

To find some reason for the variation in the distribution of perithecia just described, experiments were devised in which each plate of paired mycelia was unevenly illuminated and then the resulting distribution of perithecia was observed.

A. Half of each culture illuminated. Half of the lid and half of the container of each of twenty pairs of Petri dishes were painted black outside. The lids and containers were put together so that one side of each culture would be almost completely in the dark. The twenty dishes were each inoculated on opposite sides with mycelia nos. 3 and 4 in such a way that there were ten dishes with mycelium no. 3 in the blackened half and ten dishes with mycelium no. 4 in the blackened half. The cultures were grown under an electric-light bulb for six days and then examined. The distribution of the perithecia was as follows.

Of the twenty cultures, thirteen had perithecia on the lighted mycelium only; three had most of the perithecia on the lighted

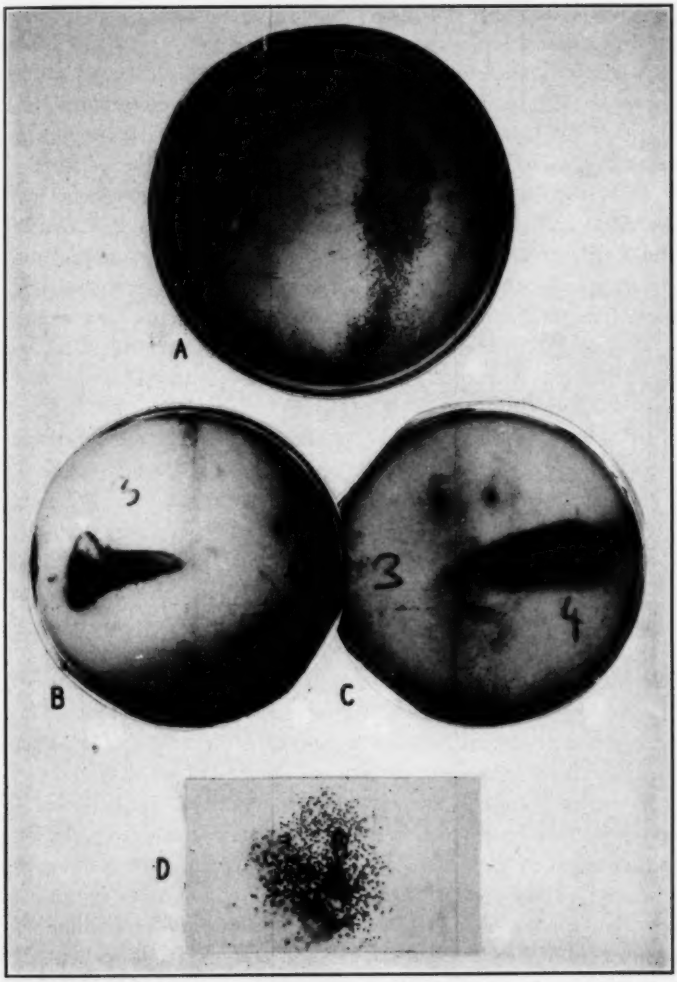

FIG. 4. *Gelasinospora tetrasperma.* Nuclear migration. *A:* mycelia nos. 3 and 4 mated; perithecia have formed on mycelium no. 3. *B* and *C:* effect of light on nuclear migration; in *B* part of mycelium no. 3 was illuminated and in *C* part of mycelium no. 4; perithecia have been formed in the illuminated parts of the mycelia. *D:* mycelium no. 3 patched with mycelium no. 4; perithecia have been formed on the agar surrounding and at some distance away from the patch of mycelium no. 4. Magnification: *A, B,* and *C,* about half the natural size; *D,* slightly less than natural size.

mycelium; three had perithecia equally on the lighted and darkened mycelia; and one was sterile.

It is evident that, in the experiment just described, the perithecia occurred most frequently on the illuminated mycelium, thus showing that light favoured their development. The darkened mycelium, whether no. 3 or no. 4, seldom produced any perithecia.

After the distribution of the perithecia in the experiment just described had been noted, transfers were taken from a darkened and sterile mycelium no. 3 and from a darkened and sterile mycelium no. 4, whose lighted mates had produced perithecia, and these transfers were grown separately in the light for two weeks. They failed to produce any perithecia and thus revealed that they had remained unisexual. Nuclei of opposite sex could not have migrated into them.

B. A small area of each culture illuminated. Four Petri dishes were each inoculated on opposite sides with mycelia nos. 3 and 4. They were then wrapped in black paper in which a small hole had been cut through which light could pass. The holes were arranged so as to be: in two of the plates over mycelium no. 3, and in the two other plates over mycelium no. 4. The plates were then placed under an electric-light bulb. At the end of a week perithecia had begun to be formed. Four days later the lids of the Petri dishes were removed and the cultures were examined. It was found that the perithecia were distributed chiefly in that part of the plate that had been illuminated regardless of whether the illuminated mycelium had been no. 3 or no. 4 (FIG. 4, *B* and *C*).

From the results of the experiments described above under *A* and *B* we may conclude that, in *Gelasinospora tetrasperma,* during the initiation of the sexual process, light influences the migration of nuclei of one sex into the mycelium of opposite sex in such a way that the nuclei tend to pass out of a darkened mycelium of one sex into the illuminated part or parts of the mycelium of opposite sex.

The direction of nuclear migration is influenced not only by light but by other conditions. This was made clear when mycelia nos. 1 and 3, which were of opposite sex, were patched in both the possible ways and under identical conditions of illumination. It was found that: when patches of mycelium no. 1 were placed on

cultures of mycelium no. 3, mycelium no. 3 soon produced perithecia around the patches; but that, when patches of mycelium no. 3 were placed on cultures of mycelium no. 1, in none of about a dozen experiments did any perithecia appear around the patches on mycelium no. 1.

VII. THE PATH OF NUCLEAR MIGRATION

Two plates were inoculated, one with mycelium no. 3 and the other with mycelium no. 4. Then the plates were bridged by means of a strip of cover-glass 2 mm. wide in the manner shown

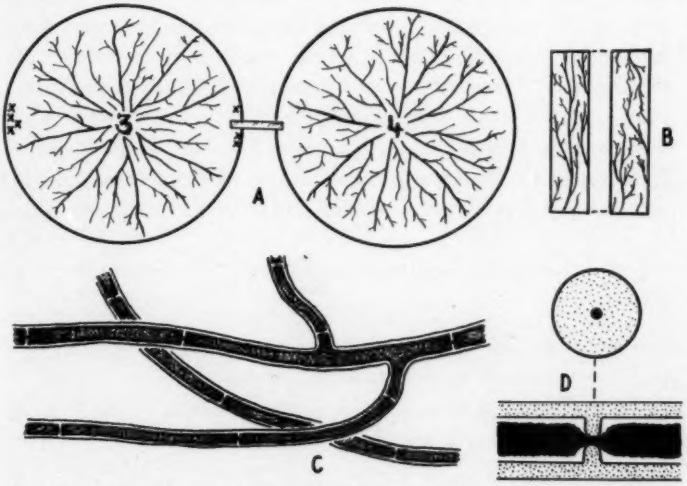

FIG. 5. *Gelasinospora tetrasperma.* The path taken by migrating nuclei. Explanation in the text.

in figure 5, *A*. At the end of six days the two mycelia had covered the agar and about twelve hyphae had grown on to the bridge (FIG. 5, *A* and *B*). The bridge was then removed and the plates were separated. Six days later perithecia appeared in the dish containing mycelium no. 3 in the positions shown in figure 5, *A* by means of crosses.

In the meanwhile, the cover-slip bridge (FIG. 5, *B*) was mounted on a slide and the hyphae upon it were treated first with iodine in potassium iodide to stain the protoplasm deep-brown and after-

wards with chlor-zinc iodine to swell the cell-walls (**2**, pp. 89–91). It was then found that, as already described by Buller for the mycelia of Pyrenomycetes and Discomycetes in general (**2**), every hypha on both surfaces of the bridge had a protoplasmic strand passing from one cell to the next through the central pore of each septum (FIG. 5, *C* and *D*). It was also observed in studies of the living hyphae of *Gelasinospora tetrasperma* that each new septum is formed near the end of an elongating hypha as an annular ingrowth from the lateral wall and that it completes its development, with reduction of the open pore to the minimum width, in about thirty minutes. It must therefore be admitted that, in the experiment illustrated in figure 5, *A*, nuclei which passed from mycelium no. 4 across the cover-glass bridge into mycelium no. 3 must have passed through the septal pores actually shown, or like those shown in figure 5, *C* and *D*. This is the first time that experimental evidence has been obtained that proves conclusively that migrating nuclei in an Ascomycete are able to pass *via* septal pores from one cell to the next.

VIII. NUCLEAR MIGRATION AND CYTOPLASMIC STREAMING

It has been shown by Buller (**2**) that, in the Pyrenomycetes, Discomycetes, and Hymenomycetes, as a hypha elongates, cytoplasm is constantly flowing toward the growing point. Thus the growth of a hypha is in part due to the extension of the cell-wall at the tip of the hypha and in part due to the newly created space in the terminal cell of the hypha being constantly filled up by cytoplasm that flows to the tip of the hypha from the column of cytoplasm in the terminal and subterminal cells.

In *Gelasinospora tetrasperma,* with the low power of the microscope, it can readily be seen that the column of cytoplasm in the terminal and subterminal cells of a growing hypha is continuously flowing toward the growing point, and it was found that this flow can be beautifully demonstrated by means of a moving-picture film.

In *Gelasinospora tetrasperma* the column of cytoplasm that flows toward the tip of each growing hypha passes from one cell to the next by way of the central perforation in each septum, *i.e.* in the

same manner as that observed by Buller (2) in Pyrenomycetes, Discomycetes, and Hymenomycetes in general.

In *Gelasinospora tetrasperma,* in a subterminal cell exhibiting streaming, one gains the impression that all the protoplasmic contents are streaming with the exception of a vacuole which is frequently pressed against the distal septum. This question therefore arises: are or are not the nuclei carried in the column of streaming cytoplasm from one cell to the next as it flows toward the hyphal tip? If the nuclei are carried in this way, the younger cells near the hyphal tips should contain more nuclei than the older highly vacuolated cells of the mycelium from which the massive part of the cytoplasm has already flowed. To compare the number of nuclei in young and old cells of hyphae exhibiting streaming, we proceeded as follows.

Two Petri dishes, A and B, each containing a glass slide, were filled with agar so that the slides were just immersed, and they were then inoculated with a mycelium of *Gelasinospora tetrasperma.* In dish A the inoculum was placed some distance away

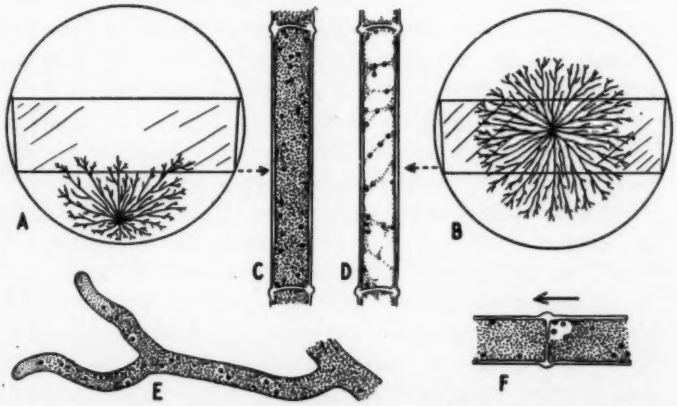

FIG. 6. *Gelasinospora tetrasperma.* The nuclei in the cells remain fixed while cytoplasm flows toward the tips of the growing hyphae. Explanation in the text. Magnification: *C–F,* 575.

from the slide, while in dish B the inoculum was placed upon the middle of the slide (FIG. 6, *A* and *B*). After about twelve hours the plates were examined. In dish A the mycelium had reached

the slide and four hyphae had grown on to it for a distance of about 1 cm. Every main hypha exhibited cytoplasmic streaming toward the hyphal tip. All of these hyphae were completely filled with protoplasm or contained a few small vacuoles. In dish B the mycelium had covered the slide and grown out over the agar beyond it for a distance of several centimeters. The main hyphae on the slide showed rapid cytoplasmic streaming toward the hyphal tips. All the older parts of the hyphae were highly vacuolated and showed only a few cytoplasmic strands remaining in each cell.

The two slides (FIG. 6, *A* and *B*) were fixed and stained to show the nuclei and then cut away from the rest of the agar in the dishes. The preparations were then mounted in glycerine.

For comparison, two typical cells approximately equal in length and in diameter were chosen, one on the slide of culture A (FIG. 6, *A*) and the other on the slide of culture B (FIG. 6, *B*); and then their nuclei were counted. In the comparatively empty cell of culture B (FIG. 6, *D*) there were 25 nuclei, while in the densely filled cell of culture A there were 22 nuclei (FIG. 6, *C*). Also observations made on many other cells on the slides of cultures A and B showed that, although the number of nuclei in a cell was distinctly variable, in general there were no fewer nuclei in old empty cells than there were in young densely filled ones.

The hyphal tips were also examined and they were found to contain nuclei which were at about the same distance from one another as were the nuclei in the vacuolated cells (FIG. 6, *E*).

A characteristic type of young cell on the slide of culture A was one densely filled with protoplasm with the exception of a vacuole occupying about half the diameter of the hypha and pressed against the septum (FIG. 6, *F*). Such a cell was found to contain about twenty nuclei, and four or five of these could be seen in the extremely thin lining layer of cytoplasm surrounding the vacuole where, from our experience of living material, there is no streaming (FIG. 6, *F*).

From the foregoing observations we may conclude that, when cytoplasm is being evacuated from older cells in a mycelium and is passing to the hyphal tips, the nuclei of the older cells are not carried away in the cytoplasmic stream, but remain anchored to the cytoplasm which forms a very thin layer that is pressed close

against the cell-walls and forms the bounding layer of the vacuoles.

That, in a cell through which cytoplasm is rapidly streaming, the walls of the vacuoles are fixed and do not flow with the stream was discovered by Buller (1, p. 129) who observed that, in *Pyronema confluens*, tiny solid bodies (his *Woronin bodies*) embedded in the walls of the vacuoles remain at rest while the cytoplasmic stream flows past them.

The mechanism which enables nuclei to migrate from a mycelium of one sex into that of another sex in such a fungus as *Gelasinospora tetrasperma* is at present unknown. There is at present no reason to suppose that the migration is dependent on the mass movement of cytoplasm which normally takes place in all growing hyphae and is always toward the growing points.

In *Gelasinospora tetrasperma*, nuclei of mycelium no. 4 must have moved clear across the mycelium no. 3 shown in figure 5, *A*; for, otherwise, perithecia could not have formed in the positions shown by the crosses; and in the Hymenomycete, *Coprinus lagopus*, Buller found that nuclei of one sex can migrate many centimeters across the middle of a large haploid mycelium of opposite sex (1, p. 233). To discover what causes nuclear movement during the migration of nuclei under conditions such as those just described is one of the tasks of the future.

SUMMARY

1. In *Gelasinospora tetrasperma*, one of the Pyrenomycetes, the migration of nuclei from a mycelium of one sex into a mycelium of opposite sex has been confirmed.

2. Migrating nuclei have been found to travel through a mycelium at a speed of from 4 mm. to 5 mm. per hour.

3. It was shown that two mycelia of opposite sex, on coming into contact on an agar plate, fuse with one another but do not overgrow one another and that the speed of nuclear migration may be twice or three times the rate at which living hyphae grow in length.

4. Light influences the migration of nuclei. In mating experiments it was found that nuclei move from a darkened mycelium of one sex toward and into an illuminated part of a mycelium of opposite sex.

5. Migrating nuclei move from cell to cell *via* the minute central pore in the transverse septum.

6. In *Gelasinospora tetrasperma,* as in other Pyrenomycetes, columns of cytoplasm are constantly flowing toward the tips of the growing hyphae. When cytoplasm is being evacuated from older cells in a mycelium and is passing toward the growing hyphal tips, the nuclei of the older cells are not carried away in the stream of cytoplasm but remain anchored to the thin fixed layer of cytoplasm that is pressed against the cell-wall and that forms the bounding layer of the vacuoles.

7. The mechanism which enables nuclei to migrate from a mycelium of one sex into and along a mycelium of opposite sex is at present unknown.

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NOTES ON GYMNOSPORANGIUM CUPRESSI

W. H. Long and L. N. Goodding

(with 1 figure)

This paper describes the pycnial and aecial stages of *Gymnosporangium Cupressi* on *Amelanchier mormonica* (?), gives an emended description of the telial stage and new data on hosts and distribution.

The telial stage of this rust was discovered by Goodding at Snebly Hill near Sedona, Arizona, in 1919 and described in 1921.[1] Every year since finding this rust both authors have searched for the aecial stage but without success until the year 1939.

In January 1937, Ranger David J. Stouffer of the Forest Service found the telial stage of this *Gymnosporangium* at Webster Springs (formerly Sawmill Springs) which is about 20 miles north of Clifton, Arizona. The galls of the rust were very common over this area, much more so than in the type locality near Sedona, for this reason the Webster Springs area was chosen during the past few seasons for intensive search for the aecial stage. Another reason for favoring this locality was the fact that junipers are not abundant in this area. No junipers occur within a half a mile radius of the place where the aecia were finally located. The authors realize that rusts do not respect such boundaries and that this aecial infection might be due to a *Gymnosporangium* on a juniper. The probability, however, of its being caused by *G. Cupressi* is much greater. Below Webster Springs, a line of heavy infection on *Cupressus* occurs along the sides of a deep draw or ravine. A second line of heavy infection follows a side draw. These two lines of infection seem to follow natural air channels which converge at a point in the main ravine where the infected *Amelanchier* was found. Adjacent to the *Amelanchier* bushes is a 15-foot *Cupressus* tree plastered with galls on twigs and small branches. So heavy was this infection that many of the twigs

[1] Long, W. H. Notes on new or rare species of rusts. Bot. Gaz. **72**: 39–44. 1921.

were killed. It, therefore, seems reasonable to assume that the aecia discovered on the *Amelanchier* leaves are those of *G. Cupressi;* furthermore the characteristics of these aecia do not correspond to any known species of *Gymnosporangium.*

The type description of this rust gives *Cupressus arizonica* Greene as its host; later investigations show that the host is *C. arizonica* var. *bonita* Lemm. (*C. glabra* Sudw.) since this is the only species growing in the Sedona area (type locality). The *Cupressus* at Webster Springs is the true *C. arizonica,* in fact this general area is the type locality for this species. This makes two hosts for the telial stage of this rust—*C. arizonica* and *C. arizonica* var. *bonita;* also two widely separated localities—the Sedona area in Coconino County and the Webster Springs area in Greenlee County (formerly a part of Graham County). This *Gymnosporangium* probably occurs on these two hosts throughout their range where associated with species of *Amelanchier.*

GYMNOSPORANGIUM CUPRESSI

O. Pycniis epiphyllis, minutis, 150–200 microns latis.

I. Aeciis hypophyllis, cylindraceis, 3–5 mm. altis, 0.2–0.3 mm. latis, ad apicem non vel vix ruptis, tandem ad latera longitudinaliter fissis; aeciosporis globosis vel angularis, 16.8–18.6 × 21–24.8 microns, episporio 3–4 microns crasso, poris germantionis 5, sparsis.

O. Pycnia epiphyllous, gregarious, on pallid spots, rather conspicuous, becoming blackish-brown, globoid, 150–200 microns in diameter.

I. Aecia hypophyllous on thickened discolored spots, few, solitary or in small groups, cylindrical, 3–5 mm. high by 0.2–0.3 mm. in diameter; peridium white (hyaline), tardily if at all dehiscent at apex, slowly rupturing by longitudinal slits along the sides; peridial cells usually seen in face view, not hygroscopic, remaining straight when wet, linear-oblong to oblong-lozenge-shaped in face view, 16–18 × 62–93 microns, linear-oblong to oblong in side view 20–27 microns thick, the outer wall thin, 1–1.5 microns, smooth, the inner and side walls 5–7 microns thick, closely verrucose-rugose with irregular ridge-like papillae; aeciospores angular to globoid, 16.8–18.6 × 21–24.8 microns; walls chestnut-brown, 3–4 microns thick, very minutely and closely verrucose-rugose, appearing almost smooth, germ pores large, evident, 5, scattered.

On Malaceae. Collected on *Amelanchier mormonica* (?) C.

Schneid., Webster Springs, 20 miles north of Clifton, Arizona, on Coronado Trail, October 6, 1939, by *Leslie N. Goodding* and *T. D. Mallery* (No. *8439* Long) at 7,000 ft. elevation.

III. Telia caulicolous, from a perennial mycelium, appearing on twigs, branches and trunks, causing fusiform to subglobose galls

FIG. 1. *Gymnosporangium Cupressi.*

with bark much roughened and exfoliating (FIG. 1), 0.4–90 cm. long by 0.5–30 cm. thick, usually breaking forth irregularly and often transversely on the smaller branches and twigs, in irregular rows in deep longitudinal fissures of the bark on the larger branches and trunks, emerging in oval to elliptical sori, 0.5–2 × 1–5 mm., at first partially covered by the rupturing bark, later this bark may fall away entirely leaving the sori naked, or the bark may persist as an irregular cup around the sori, telia at this stage flat, firm, chestnut-brown, when mature, more or less tongue-shaped, often irregular and somewhat crenate at top, before gelatinizing 2–10 mm. broad by 4–6 mm. tall, dark chestnut-brown, becoming cinnamon-brown after expansion; teliospores 2-celled, spores with col-

ored walls oval to ellipsoid, 20–27 × 30–50 microns, slightly or not at all constricted at septum, the two cells subequal; walls 2–3 microns thick, fulvous, germ pores two in each cell near septum; pedicel solid, hyaline, cylindrical, 3–4 microns thick, 100–175 microns long; teliospores with thin, colorless walls, oblong to narrowly ellipsoid, not constricted at septum, 16–20 × 40–60 microns, the two cells subequal, spores rounded at both ends, germ pores two in each cell at septum, walls 1–1.5 microns thick.

On Juniperaceae. Type collected on *Cupressus arizonica* var. *bonita* Lemm. (*C. glabra* Sudw.) at Snebly Hill near Sedona, Ariz., May 26, 1920, by *Leslie N. Goodding* (No. *6906* Long); also same host and locality in 1919 (No. *6903* Long); on same host on Cottonwood-Sedona road, 6 miles from Sedona, May 26, 1920 (No. *6904* Long).

On *Cupressus arizonica* Greene near Webster Springs about 20 miles north of Clifton, Ariz., January 12 and March 13, 1937, by *David J. Stouffer* (Nos. *8052* & *8092* Long); on same host and same locality, May 7, 1937, by *Leslie N. Goodding* (No. *8192* Long).

DEVELOPMENT OF THE CARPOPHORES OF CERTAIN BOLETACEAE

R. P. Elrod[1] and Walter H. Snell

(with 2 figures)

The available information concerning the development of the carpophore of the Boletaceae is very incomplete. At present it consists of a few fragmentary statements, some speculative conclusions and only a few well worked-out studies. It is desirable that the facts be obtained because in the past some taxonomic arrangements of the higher fungi have been based fundamentally upon the type of development of the carpophore and because such distinctions may be of contributory, if not fundamental, importance in the classification of the Boletaceae. This paper adds studies of three species of *Boletus* in the group Viscipelles of Fries and Peck (genus *Ixocomus* Quélet of the Europeans) and two species of *Boletinus*.

DeBary (**1**, pp. 287–290) stated that *B. luteus* L. ex Fries, *B. elegans* Fries and their relatives are angiocarpic because of marginal veils, while other species are gymnocarpic. J. Schroeter (**8**, p. 62) placed the Boletaceae in his Hemiangiocarpeae. P. Hennings (**4**, p. 108) stated that certain of the species of this family have their tubes exposed from the beginning (hence, are gymnocarpic). Patouillard (**7**, p. 34) divided his "Basidiomycètes homobasidiés normaux" into gymnocarpic "Aphyllophoracés," hemiangiocarpic "Agaricés" (in which the Boleti and Paxilli were included as the Boletés), and the angiocarpic Gasteromycetes, fundamentally on the basis of the characters as stated.

In a complete study of *B. Zelleri* Murr., Zeller (**10**) showed that the first development is a cleavage plane from the outside inward, which forms an annular furrow. A palisade then de-

[1] A large portion of the material presented in this paper formed a part of the thesis presented by the first author in partial fulfillment of the requirements for the degree of Master of Science in the Department of Botany in the Graduate School of Brown University, May, 1938.

velops on both the upper and lower surfaces of this furrow. Hence, this species is gymnocarpic.

Yates (**9**, pp. 229 and 234) made the statement that his incompleted study of *B. granulatus* L. ex Fries confirms the conclusions of Zeller with *B. Zelleri.* He adds that he has found a definite veil in young plants of *B. edulis* Bull. ex Fries and *B. chrysenteron* Bull. ex Fries 10–15 mm. high, but in both cases the veil entirely disappears, leaving no trace either of annulus on the stipe or of fringe on the margin of the pileus. No details were given by Yates for either of these situations. One is left to conclude that he referred, not to a universal veil in any sense, but to a marginal veil formed by the outgrowth of the incurving margin of the pileus, which reaches and makes contact with the stipe, as found in other species by Kühner and by the authors of this paper.

Kühner (**5**) found that *B. parasiticus* Bull. ex Fries is gymnocarpic, with a development very similar to that of *B. Zelleri.* On the other hand, in the annulate *B. flavus* With. ex Fries (*B. elegans* Fries?), in the very youngest stages, as the pileus enlarges and as the stipe is forming a true hymenium with basidia and cystidia, the margin tends to incurve. As further enlargement takes place, the margin soon makes contact with the hymenial surface of the stipe, producing inside this false veil a cavity in which the hymenium shortly is formed. He called this type of development pseudoangiocarpic, rather than hemiangiocarpic. Kühner also states that *B. viscidus* and probably all annulate species of *Ixocomus* are similarly pseudoangiocarpic. In a later paper (**6**), he stated that *Gyrodon lividus* (Fries ex Bull.) Sacc. is gymnocarpic and he showed that *Boletinus cavipes* (Opat.) Kalchbr. is pseudoangiocarpic like *Boletus flavus,* with a development similar to it except that in the former only the upper portion of the stipe is fertile instead of the entire length as in *B. flavus.*

Gilbert (**3**), in his characterizations of the genera of his order Boletales, included the following descriptive terms: *Boletinus* at first gymnocarpic, then pseudoangiocarpic; *Ixocomus*—gymnocarpic, sometimes pseudoangiocarpic; *Boletellus* and *Strobilomyces* —probably gymnocarpic, then pseudoangiocarpic; *Paxillus, Phylloporus, Xerocomus, Boletus, Krombholzia, Porphyrellus, Gyroporus, Gyrodon* and *Boletinellus*—all gymnocarpic.

BOLETUS PLACIDUS BON.

In the smallest specimens of this species examined, 250–300 μ broad and 2.8 mm. tall (FIG. 1, *A*), a pileus of irregular shape is already present, often somewhat broader than the stipe portion and with a slight constriction at the apex of the stipe. The tissue of this primordium of the pileus is undifferentiated, but there are loose projecting hyphae on the margin. The stipe already has a palisade on its surface and glandular dots of caulocystidia.

In the 500 μ stage (FIG. 1, *B*), there is considerable constriction of the apex of the stipe, and the pileus, although still irregular in shape, has become more like the normal form of mature specimens. The upper portion of the pileus flesh has become somewhat looser than the remainder and there is a beginning of a dermal layer of overlapping hyphae. On the lower side of the pileus, ends of hyphae have bent down perpendicular to the surface to form a palisade. No change can be noted in the stipe tissue.

In the 650–700 μ stage, 2.5 mm. tall (FIG. 1, *C*), there is no constriction of the apex of the stipe. The pileus is still irregular in outline, with a margin very much incurved toward the stipe, and the marginal hyphae, appearing like a brush in the sections, extend nearly to the surface of the stipe. The surface layer of the pileus has become slightly compacted. On the stipe, the only change is that the clusters of the cystidia have become more abundant.

In the stage 1.36 mm. broad and 3 mm. tall (FIG. 1, *D*), the pileus has become regular in shape, and the margin is still incurved, but the marginal hyphae have not yet reached the stipe. There has been no change in the flesh, but there is a surface layer of more or less erect, slightly tangled hyphae 110–145 μ long, 3–3.5 μ thick, which does not extend quite to the extreme margin. The hymenium is now compact and fairly well formed. It has no mature basidia, but has a few isolated cystidia and a few glandular dots of cystidia near the stipe. The stipe itself is thickly glandular-dotted two-thirds to the base and the lower portion of its flesh has become looser in the center.

In the 1.6 mm. stage, 5 mm. tall (FIG. 1, *E*), the marginal hyphae have made contact with the stipe in a compact mass, but have not

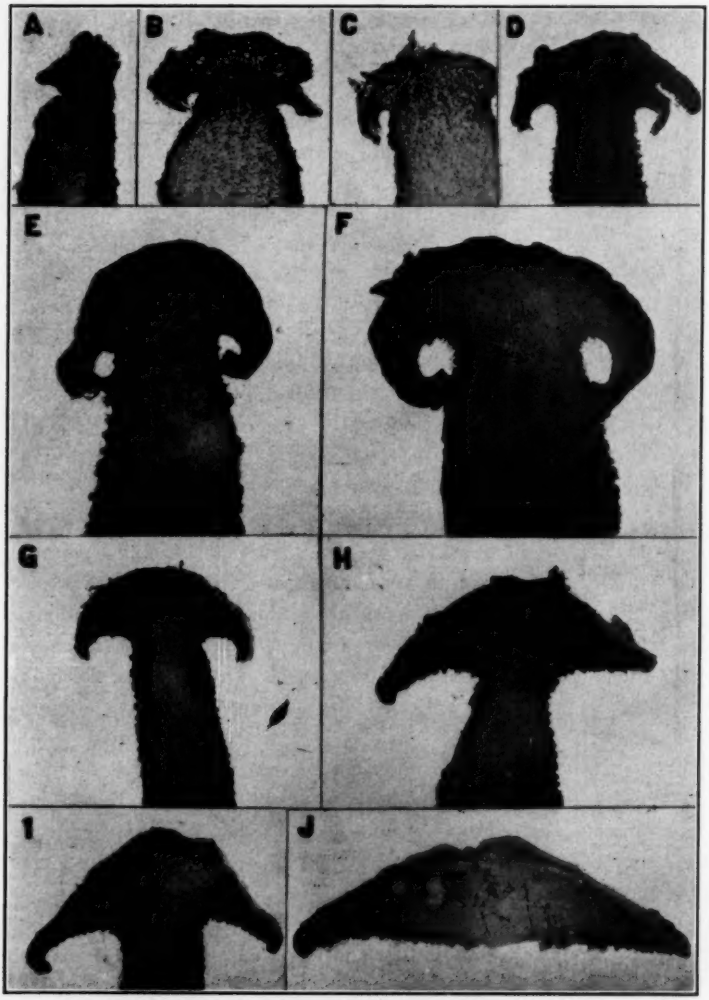

FIG. 1. Development of the carpophore of *Boletus placidus*. *A*, first stage of pileus evident, 250–300 μ broad, and stipe with a palisade on the surface and glandular dots and caulocystidia, × 50. *B*, 500 μ stage, showing evidence of a dermal layer, with incurved, hairy margin and palisade of parallel hyphae on lower side of pileus, × 50. *C*, 650–700 μ stage showing little advance except the increasing prominence of the glandular dots of the stipe, × 50. *D*, about 1.4 mm. broad, with primordial ixotrichoderm on

at all become fused with it. The surface layer of more or less erect hyphae now extends entirely to the beginning of the outgrowth of marginal hyphae and is 160 μ thick on top, but thinner toward the margin. The hyphae are now less distinct because they have become partially gelified. For a covering of this type, which ultimately makes a viscid, viscous or glutinous surface in many Boletaceae and which is probably of the same sort in agarics, if not in other groups, the second author has been using the term "ixotrichoderm" (2, p. 699, footnote 2). Beneath this layer of gelifying hyphae, extending from the margin inward nearly to the flat dome of the top of the pileus there is a narrow, darker-appearing zone of compacted hyphae which has the appearance of a cutis, but which from its position probably should be termed a subcutis. The hymenial palisade now extends half way to the margin, and has more cystidia, and a few matured basidia bearing spores. There is no change in the condition of the stipe.

In the next stage, with the pileus about 2.5 mm. broad and 5.5 mm. tall (FIG. 1, *F*), the only noticeable changes are as follows:—the tissue of both pileus and stipe is more loosely tangled; the hymenium has glandular dots all over its surface; and the stipe is glandular-dotted to the base.

In the next stage, about 3 mm. broad and 7 mm. tall (FIG. 1, *G*), the pileus is partially expanded. The margin has pulled away from the stipe, is now somewhat curved but practically vertical, and there are no signs of the marginal hairs on the stipe to form any semblance of an annulus. The ixotrichoderm has a somewhat different appearance. In the layer of gluten are hyphae that appear fresh and new because of their sharpness of outline, regularity and deep-staining quality. The suggestion is that the first hyphae of the layer became completely gelified and that new ones have grown up from the compacted layer (which now completely covers

surface, well-developed hymenium and prominent glandular dots on the stipe, × 25. *E*, 1.6 mm. broad, with ixotrichoderm partly gelified and marginal hyphae in contact with the stipe forming a chamber around the developing hymenium, × 25. *F*, 2.5 mm. stage showing marginal hyphae appressed to stipe but not fused with it, × 17. *G*, about 3 mm. broad, with margin pulled away from the stipe and no sign of marginal hairs or of annulus, × 12. *H*, 3.5–4 mm. broad, with pileus farther expanded and hymenophore somewhat undulated, × 12. *I*, about 5.4 mm. broad, × 8. *J*, pileus 8 mm. broad, × 8.

the pileus) to continue the production of gluten. The hymenium is seemingly more compact and more chromophilous, with abundant glandular dots, but still with few mature basidia. The stipe has changed only to the extent that the flesh has become longitudinally areolate and looser toward the base.

In the stage 3.5–4 mm. broad and 11 mm. tall (FIG. 1, *H*), the pileus has become expanded into its mature shape, although the margin is somewhat curved, and wadded or tufted. The condition of the ixotrichoderm is about the same, in that no further gelification is evident. The hymenophore has begun to undulate, the folds covered with palisade are about 50 μ high, and the glandular dots are prominent. In the stage about 5.4 mm. broad (FIG. 1, *I*), the only change is the somewhat greater height of the hymenial folds (65 μ), but in the 8 mm. stage (FIG. 1, *J*), the pileus has become expanded, any marginal hyphal wad has disappeared and the tube-wall primordia are 1.5 mm. long.

Boletus placidus is therefore pseudoangiocarpic in development like *B. flavus* and *Boletinus cavipes*, in that the hymenophore begins its development in the open, later becomes enclosed in a cavity by the incurving of the margin of the pileus and the development of the marginal hyphae which make contact with the stipe, and then completes its development in the open as the margin pulls away from the stipe. As far as is known to the authors, this is the first reported case of pseudoangiocarpy in exannulate forms of the Boletaceae.

BOLETUS GRANULATUS AND B. AMERICANUS

The development of *B. granulatus* (FIG. 2, *A–E*) and *B. americanus* (FIG. 2, *F–H*) is in each case approximately the same as that of *B. placidus*. They both start when 250–400 μ broad to enlarge slightly at the tip of the stipe, and at about 400–500 μ the margins of both begin to curve toward the stipe, finally reach it and then separate without leaving any semblance of annular material on the stipe. Also, the development of the viscous layer on the pileus, of the glandular dots on the stipe, and of the hymenium is about the same in all three species.

In *B. granulatus*, however, the contact of the marginal hairs with the stipe occurs somewhat earlier than in *B. placidus*, when

the young pileus is 700–800 μ broad, and the margin breaks away somewhat earlier, at about the 2.3 to 2.4 mm. stage. Further, in *B. granulatus,* the hymenial palisade does not appear at the apex of the stipe until the pileus has reached about 500 μ in breadth, whereas in the other two species it appears in the 300–400 μ stages. Likewise, the first spores are not produced until a little later than in the other two species—*i.e.,* at about the 2 mm. stage instead of the 1.6 to 1.9 mm. stages in the others.

Boletus americanus differs from the other two species in only two minor respects. For one thing, in the material available, the making of contact of margin with stipe was found to be irregular. In some series of specimens, this contact has been made in stages as small as 1.15 mm. broad and in others, not until stages as large as 2.3 mm. Then again, the surface clothing in some of the younger specimens is more of an ixocutis than an ixotrichoderm—that is, the projecting surface hyphae which would later gelify to form the viscous layer are often flat and parallel to the surface rather than wavy-erect.

Both *B. granulatus* and *B. americanus* are therefore pseudoangiocarpic in development, like *B. placidus.* This was to be expected in this subgroup of closely related species with glandular-dotted stipe in the Viscipelles (or new genus *Ixocomus*). Yates' implication (*loc. cit.*) that the development of *B. granulatus* is gymnocarpic, with the hymenium forming externally in an annular groove or furrow as in *B. Zelleri,* was apparently based upon a study of insufficient material.

SUMMARY OF THE VISCIPELLES OF THE GENUS BOLETUS (GENUS IXOCOMUS QUÉLET)

Thus far, the development of four species of the Viscipelles has been studied completely—the annulate *B. flavus* (*B. elegans* ?) by Kühner (**5**) and the three exannulate species presented above. In each case, the development is pseudoangiocarpic. The same is probably true of the annulate *B. subluteus* Peck. In the few young stages of this species studied by the present writers, every feature is similar to what has been found for comparable stages of the four species just mentioned, except of course for the lack

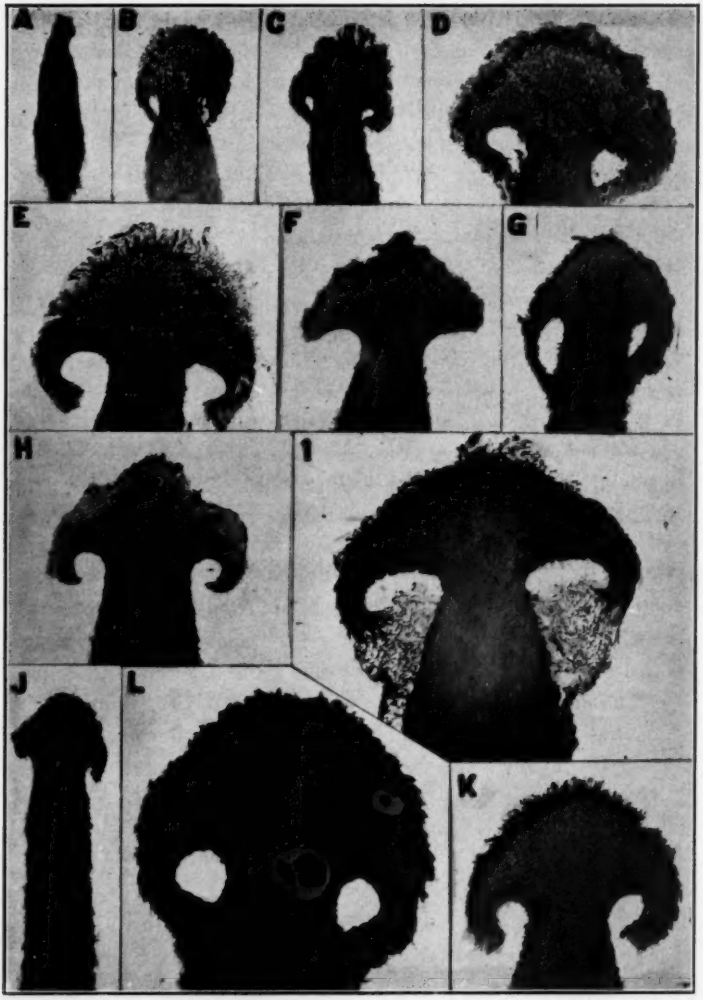

FIG. 2. *A–E, Boletus granulatus: A,* entire carpophore, 240 μ broad at tip, × 20; *B,* 560 μ stage, with marginal hyphae barely in contact with the stipe, × 20; *C,* 800 μ stage, with margin in contact with stipe and the hymenial cavity closed, × 20; *D,* 1.7 mm. stage, with pileus expanding but the margin still in contact with the stipe, × 20; *E,* 2.4 mm. stage with the margin pulled away from the stipe, × 15. *F–H, Boletus americanus: F,* early stage, 1.2 mm. broad, before the margin begins to incurve, × 23; *G,*

of annulus in three of them. It would appear that Kühner's statement (*loc. cit.*) that all annulate species of the Viscipelles probably develop pseudoangiocarpically could be modified to include *all* species of this group (or genus), annulate or exannulate.

BOLETINUS SPECTABILIS AND B. PICTUS

In small specimens of *Boletinus spectabilis* 800 μ broad, 4 mm. tall (FIG. 2, *J*), the pileus has already taken shape, with the hairy margin curved toward the stipe. The surface consists of a trichoderm of coarse, septate filaments 80 μ long on the disc and progressively shorter toward the margin. The margin soon becomes hairy-tomentose, but the formation of scales does not take place until the pileus is 3 mm. broad.

In the youngest stages, the stipe is progressively more hairy-tomentose downward, but very shortly it becomes more hairy upward, especially as the margin approaches, and finally makes contact with, the stipe.

The hymenial region begins to be compacted in carpophores 900 μ broad. In stages 1.4 to 1.5 mm. broad, a palisade with cystidia and spore-forming basidia is present on the upper stipe, and with little more enlargement of the pileus (1.6 mm. stages, FIG. 2, *K*), cystidia and basidia are present to the top of the hymenophore arch (midway to the margin). The entire hymenial area is not fertile until about the 3 mm. stage, and even then the cystidia and mature basidia are few.

As has been implied, the pileus expands in a gymnocarpic manner, with the margin becoming hairy and curving toward the stipe before the pileus is 400 μ broad. The hymenium is mature on the stipe (at about 1.5 mm. stage) before the margin makes contact with the stipe. This incurving continues, and contact is

1.5 mm. stage, with the margin in contact with the stipe and the hymenial cavity closed, showing black-stained viscous material on the margin, × 15; *H*, 1.7 mm. stage, with the margin retracted and the hymenial cavity open, × 18; *I*, *Boletinus pictus*, 4 mm. stage, with thick marginal, wad-like veil, × 12. *J–L*, *Boletinus spectabilis: J*, 800 μ stage, with beginning of incurving of margin toward stipe, × 20; *K*, 1.6 mm. stage, with the margin incurving toward the stipe, × 20; *L*, 3.2 mm. stage, with the margin in contact with the stipe; two cavities made by larvae are present, with a cross-section of a larva in one cavity and larval pellets containing ingested spores in both cavities; × 14.

sometimes made as early as the 1.4 mm. stage, at which time the hymenium is mature and producing spores on the upper portion of the stipe. On the other hand, contact of the margin with the stipe is often not made until about the 3 mm. stage, when the hymenium is mature all the way to the margin. Hence, it may be seen that the basidia are producing spores on the stipe at least as soon as the cavity is closed, and in some, if not in most, cases, it may be fertile all over before closure takes place. The least that can be said is that the hymenium is often fertile over much of the area before the cavity is closed by the incurving margin, although the folding of the hymenophore to form the tubes does not take place until after closure.

The beginning of the formation of the hymenium is therefore external in origin or gymnocarpic, and much of the early development may be so, but the carpophore soon becomes angiocarpic (FIG. 2, *L*), and remains so until the veil parts to form the annulus when the pileus is about 5 cm. broad. Accordingly, this species is pseudoangiocarpic, like *Boletus flavus* (**5**) and *Boletinus cavipes* (**6**), at least in Kühner's sense and unless one places the emphasis upon the early and incomplete maturing of the hymenium and the first spore production.

In the smallest stages of *Boletinus pictus* available (1 mm.), the entire carpophore is covered with a loose trichoderm which is rather sharply distinguishable from the flesh of the stipe but not at all so from the flesh of the pileus, and which forms a thick wad at the point where the margin touches the stipe. In 3 to 4 mm. stages, this layer of more or less erect hyphae becomes recumbent from the disc toward the margin and tends to become pulled apart and matted into scales, which are more or less definite at about the 6 to 7 mm. stages.

The marginal hyphae are in contact with the stipe in the 1 mm. stage. The margin begins to pull away when the pileus is about 3 mm. broad, but the hyphal complex which will form the veil and the annulus is a thick wad of tomentum. This becomes thicker instead of thinner and pretty well fills the hymenial cavity until the pileus becomes 15–16 mm. broad. Only then does it begin to stretch and to form a thin, velar membrane. The veil often does

not part from the margin of the pileus until the latter is nearly 4 cm. broad.

The hymenophore of *B. pictus* is first evident as a compacting of the tissue about the 2 mm. stage and a palisade is evident at about the 3 mm. stage, with cystidia and spore-bearing basidia on the upper part of the stipe and with cystidia on the top of the arch halfway to the margin. At about 3.5 mm., the hymenophore begins to fold. At the 4.5 mm. stage (FIG. 2, *I*), the hymenium is well folded in the cavity and extends down the stipe well below the attachment of the marginal hairs of the developing veil. At the 6.5 to 7 mm. stages, the hymenium on the stipe begins to break up and the elements become laid flat to form a sort of pseudocutis. The tubes are about 1.5 mm. long on the 17 mm. stage.

It is evident that the hymenium *develops* angiocarpically, but with no stages smaller than 1 mm. available, it is not at present known whether the earliest phases of hymenium development are gymnocarpic as in *B. spectabilis* (and hence of the pseudoangiocarpic type in general), or completely angiocarpic.

The annulus of both *Boletinus spectabilis* and *B. pictus* is obviously peripileic in origin.

SUMMARY

Ontogenetic studies were made of five species of Boletaceae: three exannulate species with glandular-dotted stipe of the Viscipelles of *Boletus* (genus *Ixocomus* of the Europeans)—*B. placidus, B. granulatus* and *B. americanus;* two species of *Boletinus*—*B. spectabilis* and *B. pictus.*

The development of *Boletus placidus, B. granulatus* and *B. americanus* is fundamentally the same. The hymenium first forms gymnocarpically upon the apex of the stipe; the hairy margin of the pileus gradually incurves to make contact with the stipe as hymenial development continues centrifugally; and the margin later pulls away without leaving any semblance of annular material on the stipe. These three species are therefore all pseudoangiocarpic in Kühner's sense. As far as is known, this is the first report of pseudoangiocarpy in exannulate Boleti.

The annulate *Boletinus spectabilis* develops similarly and is likewise pseudoangiocarpic.

Boletinus pictus, likewise annulate, probably develops in a manner entirely similar to *B. spectabilis,* and is probably pseudoangiocarpic, but this cannot be stated certainly because the very earliest stages of the carpophore were not available.

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A STUDY OF SEXUALITY IN SAPROMYCES REINSCHII [1]

HARLOW BISHOP

(WITH 6 FIGURES)

In the studies of sexuality in the lower fungi the pioneer work of Blakeslee (**2, 3, 4**) on the Mucorales was of especial significance since for the first time it demonstrated by experiment that in these Zygomycetes there were two distinct types of sexuality. In one, which he named homothallic, the individual mycelium (plant) comprised both sexes so that on it conjugation and the formation of zygotes regularly took place. In the other, which he named heterothallic, an individual mycelium was of one sex only, either pure male (minus), or pure female (plus), and therefore mating with a mycelium of the opposite sex was necessary for sexual reproduction. So clear cut and definite were these sexual conditions established by Blakeslee and so effective was his experimental approach that subsequent investigations in the whole field of sexuality in the lower plants were notably stimulated and influenced. Kniep (**17**) has effectively summarized the progress of this investigation up to 1929 and has pointed out the need for further study.

In another phycomycetous group, the Oömycetes, heterothallism was demonstrated by Couch (**13**) in 1926 in *Dictyuchus monosporus* Leitgeb, a member of the Saprolegniaceae. One of Couch's female strains, however, was remarkable for its possession of latent male potentialities. Another significant contribution was the discovery by Raper (**22**) in *Achlya bisexualis* Coker, of three types of sexual strains, male, female and hermaphroditic-female. Studies in the Saprolegniaceae, therefore, indicate a more complex type of sexuality than has been thus far observed in the Mucoraceae.

In another Oömycete, *Sapromyces Reinschii* (Schroeter)

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, No. 155.

Fritsch, preliminary work by Jordan in 1927 (Weston, **26**) had shown a seemingly heterothallic condition. This species, one of the Leptomitaceae, had been selected by Weston as especially suitable for a study of sexuality because its sex organs are highly differentiated and distinctive, the oögonia large and easily recognized, the antheridial branches well developed and sharply defined, while the zoöspores are uninucleate and therefore permit "isolation and the determining of sex potentialities without the possible complications of the heterocaryotic mingling of nuclei of different sexes which might handicap such studies in the Mucorales." Moreover, *Sapromyces,* as a member of the Leptomitaceae, offers an opportunity to compare the sexuality of this family with that found in *Dictyuchus* of the Saprolegniaceae.

An investigation of the sexuality of *Sapromyces* was undertaken by the writer in 1933 and although impeded by heavy schedules of teaching has been carried on ever since. The purpose of the work was to study intensively the sexual manifestations of mycelia derived from single zoöspores and hence interpretable from the standpoint of the sexual potentialities of a single nucleus. The results are presented in the following pages.

MATERIALS AND METHODS

Of eight source collections, number 5, obtained by Dr. A. G. Kevorkian in June 1933 from a sphagnum bog near Walpole, Mass., and number 7, obtained by the writer in June 1935 from a sphagnum bog near Laurelton, N. J., at the approximate location of P. H. Jordan's collections in 1926–1927 (Weston, **26**), showed in the original cultures the characteristic sexual reproduction which readily distinguished the material as *Sapromyces Reinschii* (Schroeter) Fritsch. This species has recently been reduced by Coker (**11**) to synomymy under *S. elongatus* but the inadequacy of Cornu's (**12**) characterization of *Rhipidium elongatum* in 1872 compared with the completeness of Thaxter's (**24**) detailed description of *S. Reinschii* in 1894 seems to give doubtful basis for the change. The writer therefore retains the name *S. Reinschii* in the present paper.

The remaining six collections developed abundant zoöspores but, as in the so-called "sterile" material studied by Coker (**9, 10**) and others, formed no sex organs in the original cultures.

As any exacting study of the sexual situation in a water mold such as this demands that pure cultures be derived from single zoöspores and grown on artificial media under controlled conditions, considerable time was spent in meeting these requirements.

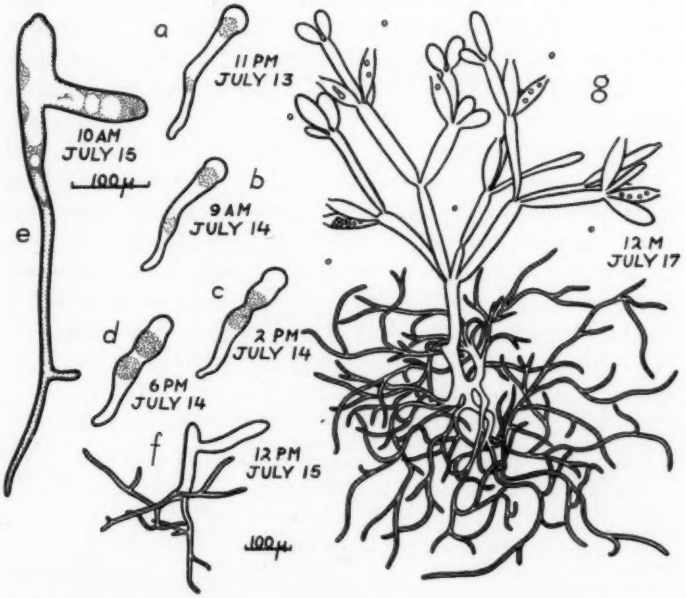

FIG. 1. Drawings showing successive stages in the development in pure culture of a single germinated zoöspore *a* into a mature plantlet *g*. From camera lucida drawings, magnification of *a–e*, upper scale, of *f–g*, lower scale.

Pure cultures from single zoöspores were obtained in two ways. In the first, from zoöspores discharged in hanging drops by repeatedly washed sporangia, young sporelings with short sturdy germ tubes were picked out with a micropipette in a Barber (1) manipulator and transferred to successive drops of sterile water until presumably free from bacteria. These selected sporelings were then transferred to drops of 2 per cent peptone solution where a satisfactory percentage of them, uncontaminated, developed in four days into vigorous plantlets with rhizoids, main axes and sporangium-bearing branches, as shown in figure 1.

As these grew larger (FIG. 1, *g*) and became visible to the unaided eye they were removed with fine forceps and planted on suitable nutrient agar in petri dishes.

In the second method, repeated isolations of vigorous hyphal tips from successive mycelia growing on prune agar and on corn meal agar finally resulted in pure mycelial cultures. These were easily induced to produce sporangia and discharge zoöspores which were picked out singly with the Barber micropipette and used to start single spore pure cultures on nutrient agar.

Twenty-three mycelial pure cultures were isolated from the sexually reproductive collections 5 and 7. From the four most vigorous and promising of these mycelia 154 single spore isolations were made, of which 37 were successfully brought to maturity to serve as stock cultures for subsequent investigation. Two more single spore cultures, isolated directly from collections 1 and 5, were successfully established. The following table (Table I)

TABLE I

ORIGIN OF THE 39 SINGLE SPORE STRAINS

Parent mycelia	Single spore strains	Totals
Sapromyces 1.....	*Sapromyces* 1C	1
Sapromyces 5.....	*Sapromyces* 5B	1
Sapromyces 5–4.....	*Sapromyces* 5T, 5W, 5X, 5L3, 5M3, 5N3, 5V3, 5W3, 5Z3, 5A4, 5C4, 5F4, 5F4-A, 5F4-B, 5J4, 5L4	16
Sapromyces 5–5–3.....	*Sapromyces* 5T4, 5X4, 5X4-D-B, 5Y4, 5Y4-G, 5Y4-H, 5Y4-I, 5Y4-J, 5Z4	9
Sapromyces 5–5–1.....	*Sapromyces* 5T5, 5U5, 5W5	3
Sapromyces 7–1–1	*Sapromyces* 7A, 7A-B, 7A-C, 7B, 7C, 7D, 7E, 7F, 7G	9

lists the sources of these 39 single spore strains, the original gross cultures being numbered 1, 2, etc., the first mycelial isolates being numbered 5–1, 7–2, etc., subsequent mycelial isolations from these 5–1–1, 5–1–2, etc., the final single spore cultures being designated by A to Z, A2 to Z2, and so on, in the order of isolation from each gross collection and without reference to the particular my-

celial partition of a gross collection (*e.g. Sapromyces* 1C was the third single spore culture isolated from gross collection 1 while *Sapromyces* 5T4 was the 98th single spore culture isolated from gross collection 5).

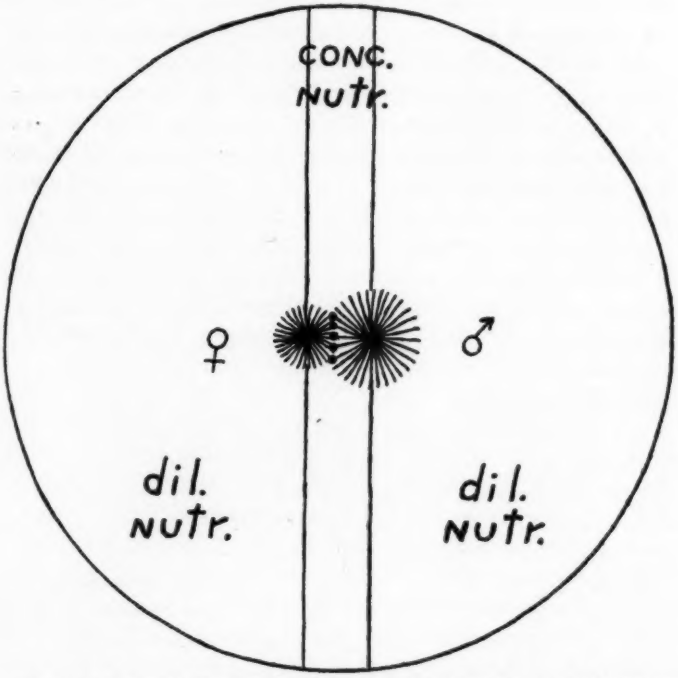

FIG. 2. Schematic diagram showing method of mating ♂ and ♀ strains in pure culture on nutrient agar in Petri dishes. The ♂ and ♀ mycelia, starting in dilute nutrient, grow across the median strip of concentrated nutrient, intermingle and develop sex organs. Mag. × 1.

Pure culture on artificial nutrient media is obviously essential in studying the sexual situation in a fungus such as this. Yet before the present investigation, no species of *Sapromyces* had ever been grown successfully thus even though von Minden (**20**) had cultured the related genera *Araiospora* and *Rhipidium* in his study of their development. The method used by Jordan (**26**) and by Kevorkian (**16**) of growing *Sapromyces* on barberries in

water cultures supported sexual reproduction and proved useful within its obvious limitations. The writer early in this investigation successfully grew *S. Reinschii* in pure culture on prune agar and corn meal agar but, on these media, although mycelial growth was satisfactory and numerous antheridial branches were produced the development of oögonia was sparse and inconsistent.

In seeking a synthetic medium which would induce the formation of abundant sex organs with certainty, the chemical analyses of barberries (**19**), prunes (**27**) and corn meal (**28**) were examined for the important nutrients common to them. It was concluded that a combination of dextrose or levulose (or both), globulin or the more easily secured proteose peptone, and such small amounts of the elements Mg, P, K, and S as are contained in Knop's solution, a mixture of the same general type as the Brown and Lipman's nitrate peptone agar of Levine and Schoenlein (**18**), should prove suitable. Accordingly several such combinations were tested and of these two proved especially successful; nutrient 1, comprising levulose 2 per cent, dextrose (anhydrous) 2 per cent, proteose-peptone 1 per cent, Knop's solution 0.1 per cent, agar 1 per cent, with glass-distilled water, and nutrient 2, the same save that the levulose was omitted. On medium 1, undiluted, both male and female strains produced dense mycelial growth but when mated neither produced sex organs since nutrient concentration of 6 per cent or higher of the three principal ingredients gave vegetative development only. On nutrient 2, both when male and female strains were mated and when they were grown separately under identical conditions, growth of the male mycelium was accelerated, of the female decelerated as is strikingly shown in figure 6, *a* and *b*, of mycelia grown in separate dishes.

Since neither these media nor their modifications were alone successful in inducing abundant and consistent production of sex organs, various combinations were tried and finally the following procedure was found to be effective. A strip of glass, one-quarter inches wide, slightly less in length than the diameter of a petri dish and of about the thickness of the layer of agar to be used, was placed flat on the bottom of a dry petri dish and the whole was sterilized. *Sapromyces* agar "1," of 1/10 dilution, but containing 1 per cent agar so as not to interfere with the hardening

qualities, was poured on the right and left sides of the glass strip and allowed to cool. The glass strip was then removed and undiluted *Sapromyces* agar "1" was poured into the slot so left. After the cooling of the entire medium, a male strain was planted at one margin of the undiluted agar and a female strain at the other margin. Within ten days the female strain formed a dense mass of oögonia and the male strain a great number of antheridial branches at the points of contact within the undiluted agar (FIG. 2).

Extensive observations of male and female strains, mated by this method, showed that functional oögonia and antheridia were formed abundantly, fertilization took place and normal oöspores were matured. Hence this method was used for most of the experimental work which follows.

EXPERIMENTAL WORK

EXPERIMENTS TO IDENTIFY SEXUAL STRAINS

The sexual identity of the 39 strains studied by the writer was at first unknown, because they had originated from single zoöspores whose derivation could not be traced with the certainty of the isolated basal cells used by Jordan in establishing the apparent heterothallism in this species.

Very early in the study, however, it became apparent that the strains fell into two groups on the basis of rate of growth of the mycelium, one group growing with noticeably greater rapidity, the other distinctly more slowly. From the findings of Blakeslee and others in their work on the Mucorales, the strains growing more rapidly would be expected to be plus or female. Yet close examination of these strains showed that frequently they produced short, slender outgrowths resembling antheridial branches (FIG. 3*b*). They were therefore considered to be probable male strains. The other less rapidly growing group of cultures never produced outgrowths resembling antheridial branches but did, on rare occasions, develop peculiar, repeatedly proliferated structures which seemed to be abortive oögonia (FIG. 3*g*). Consequently the latter strains were considered as probable females.

On the basis of the above observations ten strains (5T, 5W, 5W3, 5Z3, 5C4, 5J4, 5L4, 5T4, 5Y4, and 5Z4) were grown simul-

taneously on separate dishes of prune agar so that their growth rates could be compared. Only ten strains were tested in these early experiments since at that time only about one-half the total 39 single spore strains had been isolated. Four of these ten strains (5T, 5W3, 5Z3 and 5J4) extended 2.5 to 4.0 cm. in diameter in ten days and consistently produced outgrowths resembling antheridial branches while the other six (5W, 5C4, 5L4, 5T4, 5Y4 and 5Z4) spread only 1.0 to 2.2 cm. and one of these (5Y4) developed structures resembling oögonial initials. From these indications, strains 5T, 5W3, 5Z3 and 5J4 were tentatively considered male, and strains 5W, 5C4, 5L4, 5T4, 5Y4 and 5Z4 were tentatively considered female, and the strains of one group were mated on corn meal agar in all possible combinations with the strains of the other group. The results of these preliminary matings are shown in the following table, 0 indicating no sexual reaction and ♂♀ indicating a sexual reaction with the strain from the left hand column forming antheridial branches, the opposing strain (top column) oögonia.

TABLE II

SEXUAL REACTIONS IN THE FIRST 24 MATINGS

	5W	5C4	5L4	5T4	5Y4	5Z4
5T	0	0	0	♂♀	♂♀	0
5W3	0	0	0	♂♀	♂♀	0
5Z3	0	0♂	0	♂0	♂♀	0
5J4	0	0	0	♂♀	♂♀	0

It is of interest to note that in these matings the supposed male strains of the left column all consistently corroborated the tentative diagnosis as male, while of the supposed female strains in the top column, 5Y4 behaved consistently as female in each mating while 5T4 behaved consistently as female in three of the matings, even though in the fourth, with 5Z3, it failed to form oögonia.

In later experiments with the doubtful strains, 5W, 5C4, 5L4 and 5Z4, two of these, 5W and 5L4, proved to be neutral or neuter, since they never produced any sex organs, 5C4 proved to be practically neutral although on one other occasion it again de-

veloped antheridial branches, while 5Z4 proved to be female with the same sexual behavior as 5Y4 and 5T4, although so lacking in vegetative vigor that it became more and more feeble and eventually died.

Thus, of the first ten strains critically examined, 5T, 5W3, 5Z3, 5C4 and 5J4 were found to be male, 5T4, 5Y4 and 5Z4 female, and 5W and 5L4 neuter or neutral strains. Apparently a rough but serviceable means of diagnosis was afforded by the two criteria, *a* the rate of growth, more rapid in the male than in the female mycelia, and *b* the development of recognizable initials of the sexual apparatus, *i.e.* antheridial branches on the male and oögonial initials or abortive oögonia on the females. By means of these, the sexual character of seven out of ten cases was correctly determined, all four of the male strains, 5T, 5W3, 5Z3 and 5J4, were correctly predicted as male and the three female strains, 5T4, 5Y4 and 5Z4, as female. The two sterile strains, 5W and 5L4, and the single, weak male strain, 5C4, were erroneously predicted as female.

The strongly male and strongly female strains determined by these preliminary pairings were used in subsequent matings as the basis for identifying the sexual character of additional strains. The sexual nature of the individual isolates was not determined for all of the 39 strains but repeated tests and numerous pairings in suitable combinations revealed the character of the 17 strains most intensively studied to be that shown in the following table.

TABLE III

SEXUALITY OF THE 17 STRAINS MOST STUDIED

Strongly male strains	Weakly male strains	Neutral strains	Weakly female strains	Strongly female strains
5T	5C4	5W	5Z4	5T4
5W3		5X		5X4
5Z3		5A4		5Y4
5J4		5F4		5T5
		5L4		5U5
				5W5

The present investigation concentrated chiefly on the more strongly sexed of these strains in the hope that an understanding

of their behavior may help toward interpreting the nature of the less well defined weakly sexed strains which the writer plans to study further in the future.

MANIFESTATIONS OF SEX—GENERAL FEATURES

The distinction of male and female strains on the basis of mycelial growth being more rapid and vigorous in the male, less in the female, used in preliminary identification was found on

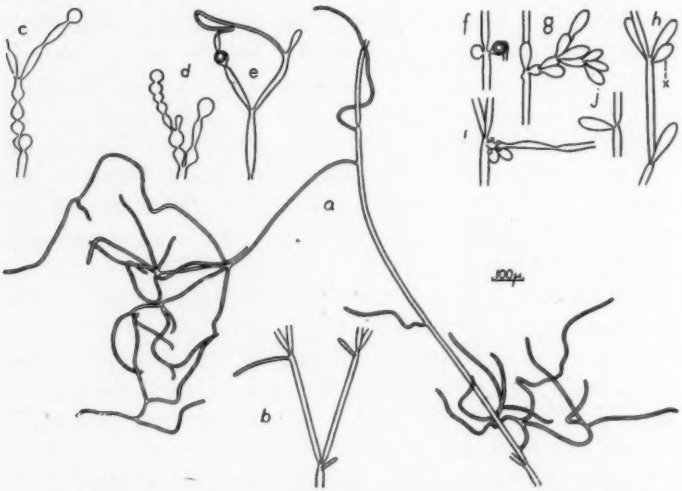

FIG. 3. Drawings showing reproductive structures developed under various conditions: *a*, antheridial branches of degree V formed by ♂ 5T mated with ♀ 5T5; *b*, antheridial branch initials formed by ♂ 5T growing alone; *c* and *d*, proliferated and aborted oögonial initials (not reached by antheridial branches) formed by ♀ 5T5 mated with ♂ 5T; *e*, hermaphroditic self-fertilization in ♀ 5T5 mated with ♂ 5T; *f–j*, sex organs of ♀ 5T4 growing alone, *f*, hermaphroditic self-fertilization, *g–j*, abortive organs. From camera lucida drawings, magnification according to the scale.

further investigation to be an even more valid and significant criterion than had at first been appreciated. Tests of various media showed that some emphasized this difference very markedly, one of these, *Sapromyces* agar 2, described above, accentuating the difference of mycelial growth so that the growth of the male mycelium in diameter was five times more rapid than that of the

female as is well shown by figure 6*a* of the ♀ mycelium and 6*b* of the ♂ after one month's growth under identical conditions.

The fact that in *Sapromyces* growth of the male strains is consistently and markedly more vigorous and rapid than that of the female is of interest as a helpful criterion of distinction between the two and because, being exactly the reverse of what Blakeslee found in the Mucorales, it casts additional light on the manifestations of sex in the lower plants.

Repeated observations revealed that the first signs of sexual reproduction consistently appeared in a zone below the apex of the segments of male and female thalli. In this zone, just below the extreme tip where the zoösporangia and vegetative segments arise, originate the first antheridial branches and oögonial initials recognizable even at early stages by their more proximal position and by the fact that they grow out more nearly at right angles. In the case of male plants the first antheridial branches appeared in this zone (FIG. 3*b*) while in response to minimal stimulation later antheridial branches also originated here although under greater stimulation they might develop at almost any point along the hyphal segments (FIG. 3*a*). In the female plants the oögonial initials (FIGS. 3*h, i, j*) usually developed in this zone under any degree of stimulation. This sub-apical area of the segment, therefore, seems to be a critical point for the formation of sex organs.

After the formation of the initials has begun the further development and maturation of the sex organs in suitably mated cultures follows a regular and well defined course. This, as is illustrated in figure 4 by four successive drawings from a complete series of camera lucida sketches of a mating of 5J4♂ with 5T5♀, involves the growth of the antheridial branch (FIG. 4*a, b*), its contact with the oögonial initial (FIG. 4*c*), the delimitation of antheridium and oögonium with fertilization by means of a fertilization tube and the maturation of a single oöspore (FIG. 4*d*). Measurements based on these drawings and on others equally typical show that the antheridial branch grew in length an average of 105 μ an hour while the oögonium increased in diameter an average of 4.2 μ an hour.

One interesting phenomenon observed repeatedly in these matings of ♂ and ♀ plants was the production of antheridial branches

by the males before any contact between the ♂ and ♀ mycelia. In matings in Petri dishes on such nutrients as corn meal agar or *Sapromyces* 1 agar with the ♂ and ♀ mycelia growing out from inoculation centers 2 to 2.5 cm. apart the ♂ plants developed antheridial branches of degree II, III or even IV (as explained later) some time before any actual contact between the approaching fringes of the mycelia. Moreover the development of antheridial branches was more extensive (degree IV) and more abundant on that part of the ♂ mycelium nearest the ♀ and decreased down to sparse (degree I) and scant development on the opposite side of the ♂ mycelium farthest from the ♀. This would seem to indicate that from the ♀ mycelium there diffuses some substance, probably a hormone, which stimulates the formation of antheridial branches by the male.

The writer, therefore, attempted under more controlled experimental conditions to secure more positive evidence for the production of such a substance by the female plants. A strongly ♀ strain 5T5 and a strongly ♂ strain 5J4 were grown on barberries in sterile water cultures in separate flasks and, after two weeks growth had covered the bottom of each flask with dense mycelial tufts, the liquid of each was filtered off through a Berkefeld filter. Meanwhile 12 Petri dish cultures had been prepared, 6 containing vigorous 4 days old ♀ mycelia of 5T5 growing on corn meal agar and 6 containing similar ♂ mycelia of 5J4. To these cultures the filtrate was added as follows: of the filtrate from the ♀ 5T5, 1 cc. was poured over the ♂ mycelium 5J4 in each of 2 Petri dishes, 1/2 cc. was poured over the ♂ mycelium 5J4 in 2 dishes and 1 cc. was poured over the ♀ mycelium 5T5 in 2 dishes. In like manner, of the filtrate from the ♂ 5J4, 1 cc. each was added to 2 dishes of the ♀ 5T5, 1/2 cc. to 2 dishes of the ♀ 5T5 and 1 cc. to 2 dishes of the ♂ 5J4.

The ♂ mycelia in the 4 dishes to which ♀ filtrate had been added in amounts of 1 cc. and of 1/2 cc. began to develop antheridial branches after 2 days. Attempts to evaluate this reaction in quantitative terms were not very satisfactory but, as a sample, an examination of 100 hyphae selected at random in a strip across the ♂ mycelium to which 1 cc. of ♀ filtrate had been added showed, after 17 days, 85 hyphae with no reaction, 7 with antheridial

branches of degree I, and 8 with antheridial branches of degree II. Yet qualitatively the experiment gave evidence for the production by the ♀ of such a substance for the ♀ filtrate did stimulate antheridial branch formation by the ♂ while in all the other plates with ♀ filtrate added to ♀ mycelium, ♂ filtrate added to ♀ mycelium and ♂ filtrate added to ♂ mycelium, no sexual reactions occurred.

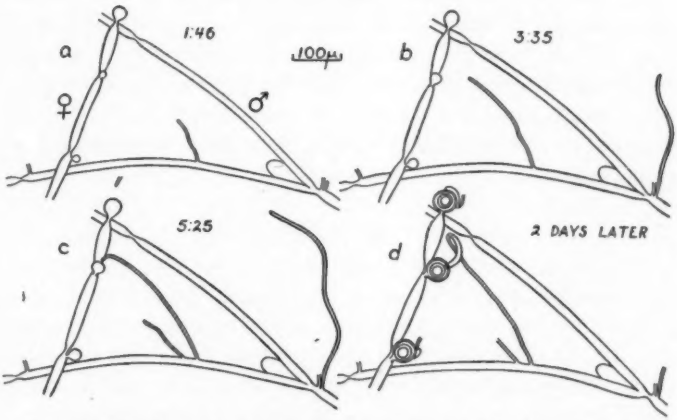

FIG. 4. Drawings showing successive stages in the formation of sex organs in a mating of 5J4 ♂ and 5T5 ♀ (under culture conditions of figure 2). Note the directional growth of the antheridial branch. From camera lucida drawings, magnification according to scale.

Another interesting phenomenon observed in matings between strongly ♂ and ♀ strains was that the antheridial branches, although somewhat sinuous rather than straight, in general appeared to grow directly toward the oögonia and, when very close, to curve precisely to attach at the very apex or distal pole of the oögonial initial (FIGS. 4, 5). Many such observations seemed to indicate that there is some definite stimulus operating to bring the antheridial branch into actual contact with the öogonium. A typical example of this phenomenon is shown in figure 6*i* in which one antheridial branch of the male strain has already made contact with an oögonium of the female strain and has developed an antheridium while three other antheridial branches can be clearly seen, their tips forming an arc of approximately 90 degrees, ac-

curately pointing toward the same oögonium. Many such observations seemed to indicate that the growth of the antheridial branches toward the oögonia was influenced by the diffusion of some substance from these.

The foregoing observations, although preliminary and not adequately verified by controlled experiments, seemed to justify the assumptions that some substance diffusing from the ♀ mycelium stimulated the development of antheridial branches on the ♂ and that these in turn were influenced to grow directly toward the oögonia by some substance diffusing from the oögonia themselves. These assumptions seem now to be established beyond doubt by the masterly work of Raper (**23**) on the rôle of hormones in the sexual reproduction of *Achlya bisexualis*.

MANIFESTATIONS OF SEX IN MALE STRAINS

The primary manifestation of sex in the male strains, namely the formation of antheridial branches, shows marked differences in degree under different conditions and in various strains. When growing by themselves on corn meal agar strongly sexed male strains always, and weakly sexed usually, form antheridial branches. These are usually short and invariably lack constrictions and secondary branches. When mated with strongly female strains on corn meal agar the vigorous male strains develop numerous antheridial axes which are of considerable length (equal to 20 hyphal segments or more) with many constrictions and abundant dense branches. Between these two extremes there are various intermediates so that it is necessary to designate by arbitrary symbols the degree of sexual response manifested. Symbol 0 denotes that antheridial branches are lacking, I that they are present but short and simple, II that they are long and simple, III that they are very long with numerous nodal constrictions, IV that they are sparingly branched, V that they are densely branched.

Whatever their degree the antheridial branches are readily distinguished from rhizoidal outgrowths. Branches arising at the critical point for sex organ formation in the zone just below the segment apex are, of course, easily distinguished by their position. Even antheridial branches arising elsewhere can be identified by their approximately uniform diameter in contrast to the tapering

of rhizoids and if of great length and profuse branching the additional feature of occasional constrictions, serving as regions of origin for the secondary branches, distinguish them from the lack of constrictions and haphazard branching of the rhizoids.

The typical behavior of strongly male strains is well exemplified by reactions of *Sapromyces* 5T. When grown alone on such media as corn meal agar or Difco prune agar this strain shows great mycelial vigor, develops abundant zoösporangia and forms small antheridial branch initials of degree I (FIG. 3*b*). When mated with strongly female strains such as 5T5 on such substrata the male develops very abundant extensive antheridial branches of degree V (FIG. 3*a*), these being so numerous and so densely matted that they can be recognized, even by the unaided eye, as a conspicuous, fine, dense turf. When mated under more natural aquatic conditions in water cultures on such substrata as barberries the male forms abundant antheridial branches of degree IV and these coming in contact with the numerous oögonia developing on the female, attach and form normal functional antheridia which accomplish fertilization.

The other strongly male strains investigated (see Table III) showed essentially the same behavior.

MANIFESTATIONS OF SEX IN FEMALE STRAINS

The typical behavior of a strongly female strain is shown by *Sapromyces* 5Y4. When grown alone on prune agar this strain develops a relatively weak mycelium with very few zoösporangia, and forms abortive oögonia occasionally and normal oögonia very rarely while on corn meal agar it commonly develops abortive oögonia and occasionally normal oögonia. In matings with strongly male strains on suitable media this strain usually produces oögonia in abundance, although occasionally few if any are formed. When, for example, this strongly female strain 5Y4 is mated on corn meal agar with the strongly male 5J4 the female develops numerous oögonia to which the antheridial branches from the male extend and the antheridium attaches to accomplish fertilization (FIG. 5*a*, *b*). When such strongly sexed strains are mated on a narrow strip of agar 1 surrounded by diluted agar (cf. Methods, Page 506) abundant production of sex organs takes place, the ♀ form-

ing dense crowds of oögonia which are consistently furnished with antheridia developed by the accompanying ♂ as is illustrated by figures *6d* and *6g* in the case of strongly ♀ 5T5 with strongly ♂ 5J4. When strongly female strains are mated with strong males

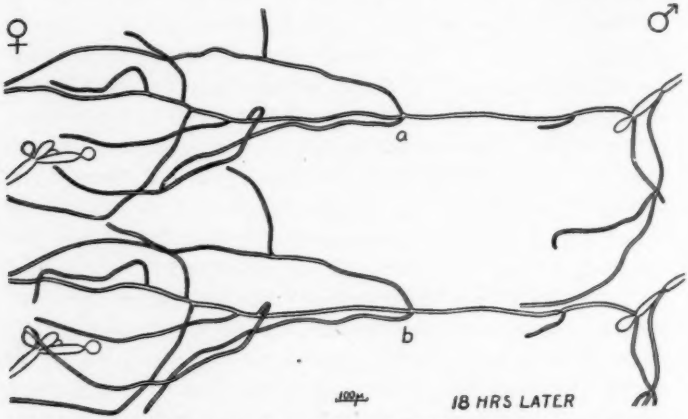

FIG. 5. Successive stages in a mating of ♂ 5J4 and ♀ 5Y4 on corn meal agar showing in *a*, the directional growth of antheridial branches toward the oögonium and in *b*, the contact, attachment and beginning of antheridium formation by one ♂ branch. From a series of camera lucida drawings, magnification according to scale.

under the more natural aquatic conditions of growth on barberries in water cultures the formation of oögonia is more profuse than on any agar medium; so abundant that it reveals itself by a dense dotting visible even under a hand lens.

Perhaps the most interesting and significant point observed in this study of sex manifestations was that the female strains, in addition to oögonia, are able to produce antheridial branches and functional antheridia. This unexpected phenomenon occurs fairly commonly in female strains grown alone in separate culture and is apparently influenced to some extent by the substratum. When grown alone on corn meal agar or prune agar, for example, the females were found to form not only oögonia but also antheridial branches (FIG. *3f*) which applied their tips to the oögonia and developed antheridia which were seemingly functional and accomplished fertilization as these oögonia developed normal mature

oöspores. In such pure cultures of females alone there could be no doubt that the antheridial branches arose from the female even though their point of origin was at such a distance from the antheridium and the oögonium to which it was attached that the course of the antheridial branch could not be traced with certainty to its source. Similarly when female strains were grown alone on barberries under the more natural conditions of water cultures these females developed not only oögonial initials but also antheridial branches and functional antheridia. Although these were slower in developing (after three weeks) and fewer in number their presence was confirmed by careful examination in numerous cultures of this kind.

When vigorous females were mated with strongly male strains on such media as a mixture of corn meal agar and half strength prune agar there occurred, in the zone of intermingling, the usual procedure of mating and fertilization as already described, the female developing abundant oögonia of which practically all are furnished with antheridia originating from the opposing male. Just outside of this zone of intermingling, however, the female not reached by antheridial branches from the male may show repeated proliferation of oögonial initials (FIG. 3*c*, *d*) with the formation of antheridial branches from the female itself. In such cases the antheridial branches originated at some distance and while in some instances it was possible to trace the antheridial branch to its origin on the female plant as much as 15 hyphal segments away, in other instances the antheridial branches were of such distant origin that their source could not be ascertained with certainty. In a few cases, however, the female developed antheridial branches so near the oögonium that the growth of the ♂ branch to the oögonium, the attachment of the antheridium and the subsequent fertilization of the oöspore could be followed with certainty. Such a case is shown in figure 3*e* where the condition of hermaphroditism with self-fertilization is obvious and somewhat resembles the androgynous condition in Thaxter's *Sapromyces androgynus* (**25**).

When similar matings were grown on strips of *Sapromyces* agar 1, surrounded by the same but diluted agar, the females were never observed to develop antheridial branches.

In none of the preceding cases did the females develop antheridial branches alone without the coincident formation of oögonia.

The foregoing instances of sexual behavior in the female strains of *Sapromyces,* involving not only the hermaphroditic production of antheridial branches but even self-fertilization, thereby in a manner comparable to homothallism in Blakeslee's sense of the term, is so different from heterothallism that it seems necessary to state that these observations were corroborated repeatedly and that all the precautions against contamination or mixing of strains emphasized by Blakeslee (**5**) as essential in the Mucorales were taken in the present work.

DISCUSSION

In order to evaluate the possible significance of the sexual condition in *Sapromyces Reinschii,* which this study has revealed, it is necessary to consider the chief theories for the interpretation of sexuality in the lower plants. Of these two are especially significant, the homothallism vs. heterothallism concept first formulated by Blakeslee (**2, 3, 4**) in the Mucorales and since elaborated and extended by other investigators (**8**), and the theory of relative sexuality formulated by Hartmann (**14, 15**) from work on the algae and since extended to other groups. Heterothallism implies strict unisexuality of ndividuals and while both plus ♀ and minus ♂ strains may show notable range in degree of sexual vigor (**6, 7**) no evidence for true sexual intergrades has been adduced. The temporarily bisexual condition in mycelia resulting from zygospore germination in forms with delayed segregation such as *Phycomyces* (**3**) or resulting from the artificial myxochimaeras produced by Burgeff (**8**) may be interpreted as instances of heterocaryosis in Burgeff's sense, the individual nuclei of the coenocytic multinucleate mycelium or of the 6 to 11 nucleate sporangial spores possibly being each of only one sexual potentiality, either plus or minus, and their relative proportions controlling the resulting sexual reactions.

Relative sexuality on the other hand postulates, even in single nuclei, male potencies (*A*) and female potencies (*G*) under the control of male realisators (alpha) and female realisators (gamma). In the male strain of haplogenotypic, heterothallic

organisms, the male realisator (alpha) is at once the means of activation of the male potency (A) and the inhibition of the female potency (G). The reverse is true in the female strains of such organisms, where the female realisator (gamma) activates the female potency (G) at the expense of the male potency (A). The strength of a given strain in sexual reproduction is termed its valence, which is the equivalent of what Blakeslee terms the "grade" or strength of sexual reaction. Most interesting of Hartmann's results are the rare copulations of strong plus with weak plus strains and of strong minus with weak minus strains. The underlying principle in his theory, then, is that each type of strain, individual, cell and even nucleus, has a bisexual potentiality.

In the light of these two theories it may be profitable to reexamine the instances of so-called "heterothallism" in the Oömycetes. The sexual strains of *Dictyuchus monosporus* studied by Couch (**13**) behaved as pure males or females with the single exception of strain "N." This female strain was parthenogenetic under ordinary conditions, but germination of one of its parthenogenetic eggs produced a strain which formed antheridia when mated with a strongly female strain. Nagai (**21**) has discovered in Japan a strain similar to this unusual one of Couch's and considers it sufficiently different to merit specific rank. At least one female strain of *Dictyuchus monosporus* thus harbors a latent maleness, which on rare occasions becomes manifest.

Coker (**10**) in 1927 described a new species, *Achlya bisexualis,* which has proved a most interesting case of intersexuality. As since investigated by Raper (**22**), this species shows the usual male and female strains, but, in addition to these, shows a curious type of strain which Raper has termed hermaphroditic-female. Its characteristics are those of pure female strains except for one feature, namely, the formation of abortive oögonia and undeveloped antheridia when grown alone. Evidently certain strains of this species have a bisexual potency.

The condition in *Sapromyces Reinschii* casts still more light on the problem of sexuality in the Oömycetes. The situation here is roughly comparable to that in *Dictyuchus* and *Achlya,* in which strains are able to form antheridia as well as oögonia. In *Sapro-*

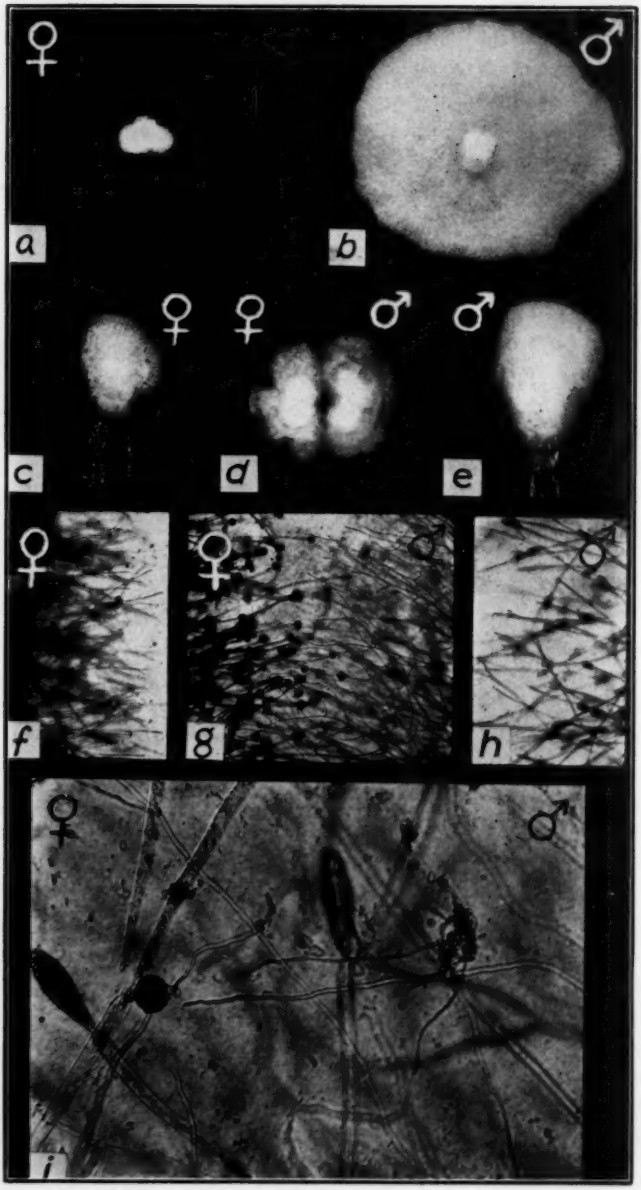

FIG. 6.

myces, however, instead of being a rare condition confined to one or a few female strains, all of the female strains carefully studied have shown that they possess a latent maleness. They may exhibit this phenomenon either when grown alone or even when mated with male strains. In common with *Dictyuchus* and *Achlya* the male strains yield only a pure male reaction in every case where they form any sex organs at all. Yet it would seem possible that suitable cultural conditions might induce some of the male strains to form oögonia for it should be remembered that at first, before suitable culture conditions were devised, the writer had great difficulty in inducing even the female strains to produce oögonia. Since all the cultures used for critical experimental work by the writer were derived from single zoöspores, and since Kevorkian (**16**) has demonstrated the presence of but one nucleus in the zoöspore of *Sapromyces Reinschii,* Burgeff's theory that each nucleus comprises the potentiality of but one sex would not explain the phenomena observed here. On the contrary the sexual reactions of these female strains indicate that both male and female potentialities are contained in the one nucleus and thus offer additional support for Hartmann's theory of relative sexuality.

Attempting to relate the results of this work with that done on related forms, the writer has formulated a possible interpretation of the sexuality of *Sapromyces Reinschii* involving six conceivable types of strains. It seems justifiable to assume that the pure male strains, symbolized by MM and exemplified by 5T, 5W3, 5Z3, 5C4 and 5J4, may have a theoretical counterpart in pure female strains, symbolized by FF. Predominantly female but latently male strains, symbolized by mF and exemplified by 5T4, 5X4, 5Y4, 5Z4, 5T5, 5U5 and 5W5, may be assumed to have a theo-

FIG. 6. Photographs illustrating typical growth and reproduction of ♀ and ♂ strains of *Sapromyces* as exemplified by ♀ 5T5 and ♂ 5J4. *a* and *b,* photographs (× 1) of ♀ and ♂ mycelia of the same age (one month) grown under the same conditions on agar No. 2 showing the notably more rapid and vigorous growth of the ♂. *c–e,* photographs (× ½) of ♀ alone, of ♂ alone, and of ♀ and ♂ mated growing on agar No. 2 according to the method shown in figure 2. *f, g, h,* photomicrographs (× 60) showing microscopic details of *c, d* and *e* respectively. Note the abundant oögonia and antheridial branches in the mated culture *g. i,* photomicrograph (× 200) (from *d*) showing the directional growth of antheridial branches toward an oögonium.

retical counterpart in predominantly male but latently female strains, symbolized by Mf. Other strains could be postulated in which male and female potentialities are nearly balanced and these might be of two sorts. In one the sexual tendencies could be strong and balanced, symbolized by MF, in the other the sexual tendencies could be weak and balanced, symbolized by mf. The latter type of strain is perhaps exemplified by 5W, 5X, 5A4, 5F4 and 5L4. Both MF and mf types might be expected to be neutral in reaction, because neither sexual tendency would predominate.

If all six types of strains, namely, MM, Mf, MF, mf, mF and FF were to be found, we would have a conveniently codified picture of sexuality in *Sapromyces Reinschii*. Of the six types of strains above postulated, only three, namely, the MM, mf and mF types, have been found as yet. The FF, Mf and MF types appear not to be represented in the strains investigated but it seems possible that collections from additional sources may yield these types. However this may be, the strains of *Sapromyces Reinschii* studied by the writer seem definitely to be of three types: pure males, predominant females with latent maleness, and neutrals.

ACKNOWLEDGMENTS

To all those who have helped to make this study possible the writer wishes to express his sincere appreciation. To Dr. William H. Weston, Jr. the deepest gratitude is felt for his suggestion of the problem and for his continued encouragement and stimulating guidance. Thanks are due to Dr. A. G. Kevorkian who supplied the writer with several gross collections of *Sapromyces*, and to Mr. Frank White who made several of the photographs which are here reproduced. While most of the work was done during the summer vacations at the Biological Laboratories of Harvard University, a considerable portion of the work was made possible through the facilities offered by Dr. P. A. Davies of the department of biology of the University of Louisville and through the many courtesies extended by Dr. J. J. Oppenheimer of that institution.

SUMMARY

1. *Sapromyces Reinschii* from several collections was isolated and grown in pure culture on various substrata, the cultural conditions favoring development of mycelia, zoösporangia and sex organs were worked out and the sexuality studied in detail.

2. Thirty-nine pure cultures originating from single zoöspores were raised to mycelial maturity. Of the seventeen isolations studied intensively, four proved to be strongly male, one weakly male, five neuter or neutral, one weakly female and six strongly female.

3. The male strains consistently showed more rapid mycelial growth than the female, this difference in growth rate being greatly accentuated on certain media.

4. When strongly male and strongly female strains were mated the former developed antheridial branches, the latter oögonia, fertilization occurred and normal oöspores developed.

5. Considerable evidence indicated that both the development of antheridial branches on the male and the directional growth of these toward the oögonia were responses to substances diffusing from the female.

6. The female strains consistently showed latent maleness and could develop antheridial branches and even accomplish self fertilization in separate culture. No comparable latent femaleness was observed in the male strains.

7. Since each strain originated from a single uninucleate zoöspore the bisexual potentiality of the females affords strong support to Hartmann's theory of Relative Sexuality.

8. Six possible types of sexuality are postulated for *Sapromyces Reinschii,* 1, pure male (MM), 2, male with latent femaleness (Mf), 3, neutral, strongly sexed (MF), 4, neutral, weakly sexed (mf), 5, female with latent maleness (mF), 6, pure female (FF). Of these possible types, only three, namely 1, (MM), 4, (mf) and 5, (mF), appear to be represented among the single spore strains investigated in this study.

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AN UNDESCRIBED FUNGUS ON JAPANESE CHERRY

E. S. Luttrell [1]

(with 10 figures)

INTRODUCTION

For the past several years Japanese cherry trees, *Prunus serrulata* Lindl., planted as ornamentals on the Duke University campus have been observed to be infected by a fungus which produces tiny, black pustules upon the surface of young twigs. Attempts at classification of the fungus and a comparison with descriptions of species reported as occurring on members of the genus *Prunus* indicate that it has not been previously described. An investigation of its morphology and development, the results of which are contained in the following account was, therefore, undertaken.

DEVELOPMENT OF THE FUNGUS

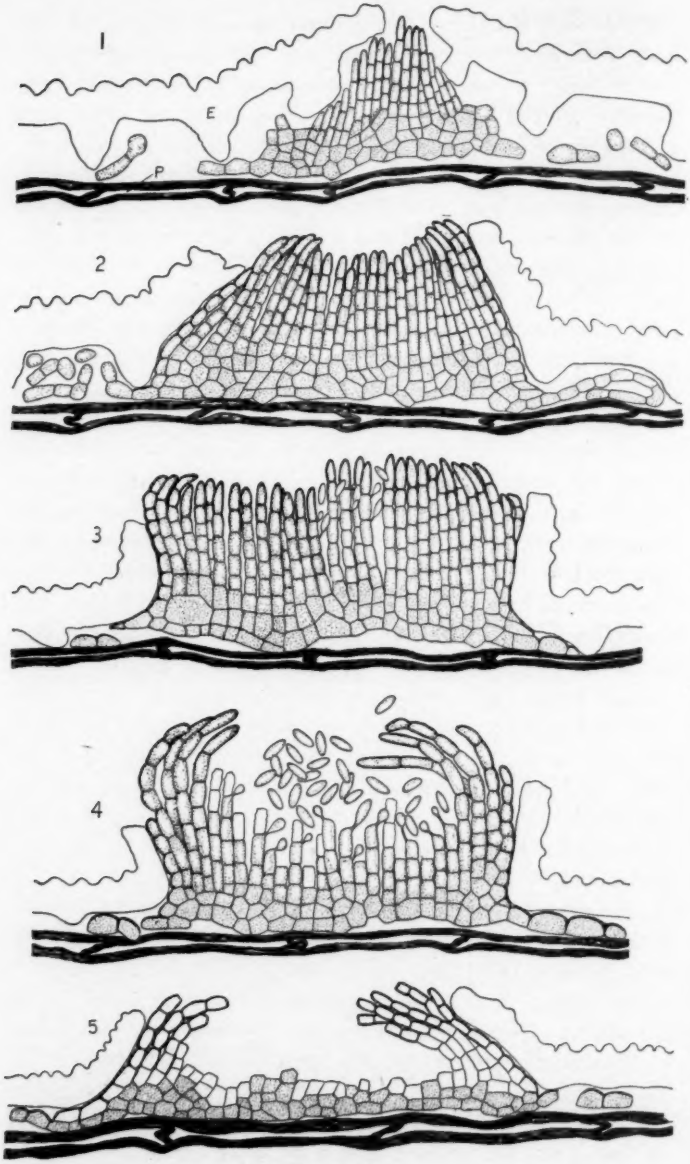
The first evidence of infection may be noted toward the end of July when black specks appear on the twigs of the current season's growth. By means of free-hand sections cut parallel to the surface, the specks are found to consist of small clusters of erect hyphae arising on the interior of certain epidermal cells. Adjacent cells contain irregular, branched mycelium which may coil and nearly fill the cell cavities. In vertical sections the mycelium is seen to be restricted entirely to the epidermis, this layer having been isolated from the subjacent tissues by periderm initiated during the preceding May. The activity of the fungus has resulted in a dissolution of the portions of the epidermal walls which lie toward the center of the stem, separating the epidermis from the underlying periderm. The mycelium now rests upon the periderm and is covered by the partially disintegrated epidermal cells and

[1] I thank Dr. Frederick A. Wolf, Duke University, Botany Department, for his advice and criticism of the work and for his material assistance in the preparation of the manuscript.

the cuticle (FIGS. 1–5). Within the lumina of some of the cells, the hyphae have become compacted into a flat, pseudoparenchymatous stroma composed of large, irregularly polygonal cells from which arise erect hyphae (FIG. 1). The hyphae are unbranched and are divided into short cylindrical cells. This structure is the young acervulus and the erect hyphae are conidiophores in an early stage of development. The tips of the conidiophores are directed against the outer epidermal wall which has been raised and is beginning to rupture in consequence of the pressure developed by their elongation. The extreme tips of the conidiophores are becoming brown and thick-walled, but the rest of the acervulus and the mycelium remain hyaline. Each cell of the mycelium and of the acervulus is filled with a dense cytoplasm and possesses a single prominent nucleus located near the center of the cell.

As the season advances, the acervuli become more prominent though by late fall they hardly exceed 100 μ in diameter. Under the hand lens they appear as black, cushion-like protuberances, flat or even slightly concave above. The acervulus by this time has enlarged to occupy the space covered by several epidermal cells. By its growth in diameter the ruptured walls and cuticle have been pushed back centrifugally to form a collar surrounding the base of the acervulus (FIGS. 2, 6). A layer of hyphae similar in structure and origin to the conidiophores but distinguished from them by their thickened walls and brown color borders the acervulus. They are sometimes longer than the conidiophores and their tips may fold inward to some extent. These hyphae never produce conidia but seem to function only as protective hyphae. The browning and thickening of the walls of the tips of the conidiophores and of the protective hyphae probably result from their exposure to desiccation. A similar response may also be observed in the mycelial cells whose outer walls become thickened and darkened, giving a pale brown color to the entire mycelium.

The earliest stages in the formation of conidia are found during late March. This process involves first the distal portions of those conidiophores located toward the center of the acervulus (FIG. 3). The conidia arise laterally. In their formation, each cell of the conidiophore produces a sterigma at whose tip is borne a globular swelling (FIG. 7). At the same time, there is a division of the



FIGS. 1–5.

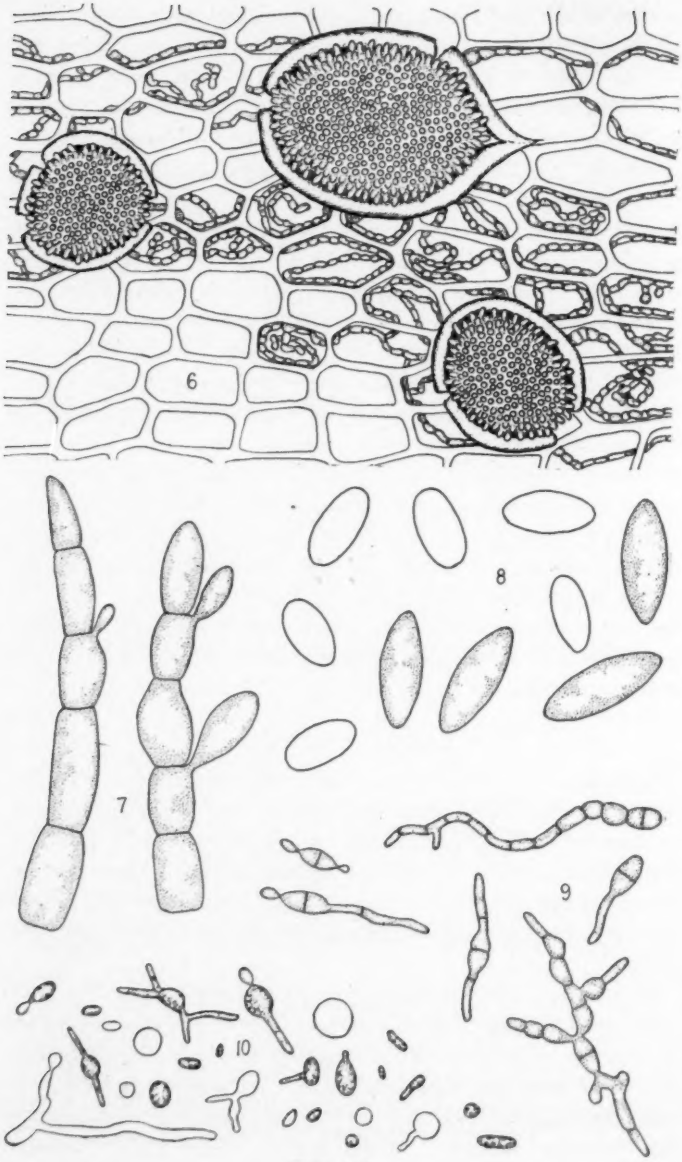
nucleus of the conidiophoral cell, one daughter nucleus remaining within the cell while the other migrates into the developing conidium, which is subsequently abstricted into the space between the conidiophores. While each cell probably repeats this process to form several conidia, it eventually becomes exhausted and disintegrates. As the distal cells are exhausted, other cells beneath them bear conidia and disintegrate in turn. The conidiophores, thus, gradually become shorter as the zone of conidial formation progresses towards the base of the acervulus (FIG. 4). As a result of the basipetal disintegration of the conidiophores, the protective hyphae bend inward and partially close the acervulus which is now filled with conidia (FIG. 4). An acervulus sectioned in this stage of development may resemble a pycnidium, and, upon superficial examination without knowledge of its development, the fungus might be mistaken for a pycnidial form.

After the conidia have been shed, the stroma and the basal portions of the peripheral protective hyphae remain as a shallow, crater-like receptacle. The rim of the receptacle gradually becomes blackened and broken, although the cells in the base of the stroma apparently contain living protoplasts until fall (FIG. 5). Further development has not, however, been observed. Disintegrating stromata may still be found within the epidermis which is being sloughed off from three year old twigs.

GROWTH IN CULTURE

Both mature and immature conidia may be obtained from the acervuli. Mature conidia are unicellular, elliptical and pale brown in color, while the immature ones are hyaline and shorter (FIG. 8). Both germinate readily either in drops of water or upon agar by the production of buds or one or more germ tubes (FIG. 9). They generally become transversely septate before germ tubes are formed.

The fungus was isolated by suspending from the top of a petri dish pieces of twigs bearing acervuli which were shedding conidia, thus making it possible for the conidia to fall upon the agar. The course of germination was followed by inverting the petri dish upon the stage of the microscope and observing the growth through the bottom of the dish. Within a few days, a powdery, white



FIGS. 6–9.

layer developed over the surface of the agar. This layer soon thickened and became wrinkled to form pale-pink yeast-like colonies. Essentially similar colonies are produced on malt agar, on agar containing shredded cherry bark, and on synthetic agar. They are composed of masses of individual cells ranging in size from 7–25 μ in diameter, and in shape from spherical to oblong or elliptical (FIG. 10). Multiplication of cells results from budding although any cell may produce one or several germ tubes from any part of its surface. The tubes rarely reach a length exceeding 100 μ, and no septations are formed. They vary in diameter from 3–8 μ, and their diameter bears little relation to the size of the cell which produced them. Living cells are filled with coarsely granular, reticulated protoplasm. Many of the cells, however, are dead, appearing as empty hyaline elements.

TAXONOMY

The fact that the fruiting body is an acervulus composed of a basal, pseudoparenchymatous stroma bearing conidiophores over its upper surface and is erumpent from the epidermal cells places the fungus at once in the Melanconiaceae. Here, because of its unicellular, elliptical, sub-hyaline conidia, it belongs in the Hyalosporae among the genera *Gloeosporium*, *Myxosporium*, *Protocoronis*, etc. From these genera, however, it differs in several important respects: (1) The conidia are produced singly on sterigmata near the apex of each cell of the simple, multicellular conidiophore. Conidial formation is initiated on the apical portion of the conidiophore and progresses toward the base. It is accompanied by a dissolution of the exhausted cells. (2) The conidiophores are surrounded by a layer of thick-walled protective hyphae which arise along the periphery of the stroma.

A new genus distinguished by the above characteristics is, accordingly, erected in the Melanconiaceae. It is named *Catenophora* in recognition of the fact that the conidiophore is composed of a series of cells each of which may produce conidia.

Catenophora gen. nov. (Etym. *catena*, chain; *phora*, bearer)

Acervulis innatis denique erumpentibus; conidiophoris simplicibus, a cellulis conidiferis constituentibus quae in catena disposuerunt atque quae seri-

atim ad basam dissiluerunt; conidiis continuis, ellipticis, conidiophorarum a lateribus orientibus, primo hyalinis, maturitate pallide-bruneolis.

Catenophora Pruni sp. nov.

Myceliis intra epidermidem; acervulis pulvinatis, atris, innato-erumpentibus, 80–160 μ diam., e hyphis sterilibus atque fertilibus efformatis; hyphis sterilibus centrifugis, flexuosis; conidiophoris (hyphis fertilibus) erectis, septatis, simplicibus, e stromate tenui oriundis, 40 × 4.5 μ; cellulis conidiferis in catenis dispositis; conidiis pleurogenis, continuis, ellipticis, primo hyalinis, maturitate pallido-bruneolis, 7–13 × 3–4.5 μ.

Hab. in ramis vivis Pruni serrulatae.

Type specimens have been deposited in the Farlow Herbarium, Harvard University, Cambridge, Mass., The Mycological Herbarium of the United States Department of Agriculture, Washington, D. C., and the Herbarium of the New York Botanical Garden, N. Y.

SUMMARY

The development of a previously undescribed Melanconiaceous fungus which infects the twigs of Japanese cherry, *Prunus serrulata* Lindl., in the vicinity of Durham, North Carolina, has been studied. The fungus was found to be distinct from other members of the Hyalosporae section of the Melanconiaceae because of its method of formation of conidia. These are borne pleurogenously on simple, multicellular conidiophores. Conidial formation involves successively the cells from apex to base of the conidiophore. The exhausted cells disintegrate. The fungus has been named *Catenophora Pruni.*

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EXPLANATION OF FIGURES

FIG. 1, section of a young acervulus of *Catenophora Pruni* in late July at the time of rupture of the epidermal wall (note the mycelium lying between the periderm (*p*) and the partially disintegrated epidermis (*e*)); 2, section of a similar stroma in November; 3, an acervulus sectioned in late March in which conidial formation has been initiated on the distal portions of the conidiophores in the center; 4, an older acervulus (May) in which the apical portions of the conidiophores have become exhausted and disintegrated leaving a conidium-filled locule partially closed by the peripheral hyphae; 5, an exhausted acervulus; 6, surface view of acervuli and mycelium of *C. Pruni* within the epidermal cells in November; 7, conidiophores; 8, conidia—the fully mature conidia have been stippled; 9, germinating conidia; 10, cells from culture.

AN ENTOMOGENOUS FUNGUS ON SPIDER MITES ON WATER HYACINTH

VERA K. CHARLES

While in Florida during the winter of 1938–1939, the author observed the frequent occurrence of spider mites, *Paratetranychus yothersi* (McGregor) [1] on the common water hyacinth, *Piaropus crassipes* (Mart.) Britton. Thinking that an entomogenous fungus might occur on these mites numerous plants attacked by them were carefully examined and as a result a fungus was found which though quite localized was evidently acting as a control. Its entomogenous character was evident from the fact that it was confined to the mites and did not spread over the surface of the leaves or in any instance penetrate the tissue of the host plant. In the natural condition it was observed that the mites were enmeshed in a fine web of white mycelium in which many individuals were dead.

The parasitism of the fungus was demonstrated by placing leaves infested by active, vigorous mites in paper bags under which condition the fungus grew rapidly on both the exterior and interior of the mites with the result that within 18 hours all the mites were dead.

DISCUSSION OF THE FUNGUS

A study of the fungus indicates that it may be considered a species of *Rhinotrichum,* a genus established by Corda (1) in 1837 and characterized by denticulate, sporiferous conidiophores and one-celled spores. Species of *Rhinotrichum* have generally been considered saprophytic though several parasitic species have been reported including two by Spegazzini, namely, *R. canescens* on *Cercospora sphaeroidea* on *Cassia occidentalis,* and *R. gossypinum* on *Cercospora Caricae* Speg. (*Asperisporium Caricae* [Speg.] Maubl.) on leaves of *Carica Papaya.* Still another species, *R. griseum* Sacc. was described on sori of *Phragmidium Rubi* (Pers.) Wint.

[1] Thanks are due Dr. H. E. Ewing for the determination of the host.

It is appreciated that the form genus *Rhinotrichum* is badly in need of revision, and that certain species now assigned to it possibly including the entomogenous species should be transferred to other genera. The suggestion has been made that it represents the conidial stage of certain basidiomycetous fungi. However, further work will have to be done to demonstrate this relationship.

In the original diagnosis of the genus *Rhinotrichum,* Corda describes the conidia as simple, but does not mention their shape, although he does in that of his type species, *Rhinotrichum simplex*.[2] Saccardo (**6**, p. 91) in the emended diagnosis of the genus describes the conidia as ovoid or oblong, hyaline, pallid or bright colored. In most of the species of *Rhinotrichum* the conidia conform to this type, but in a few species they are described as globose or subglobose.

In the majority of the species the conidia are relatively large, frequently measuring 14–15 $\times$ 22–25 μ or more and only a few species are described with conidia as small or smaller than 5 $\times$ 8 μ. Of those having smaller conidia are two entomogenous species from Ceylon described by Petch, *R. parvisporum* and *R. album* (**3**, pp. 258–9). *R. parvisporum* (**5**, pp. 243–244) was reported by Petch as occurring on scale insects of the genera *Aspidiotus* and *Lecanium*. Discussing this species he remarks that it was found in a collection of leaves of *Hevea* in which some of the *Aspidiotus* were attacked by *Pseudomicrocera* and some of the *Lecanium* by *Aschersonia marginata*. However, *R. parvisporum* was not found on scales which bore these other two fungi. The fungus was described as forming a white fringe around the scale, and spreading over it in a loose weft. This species also occurred on an effete *Gibellula* on a spider. The possible parasitism of these two entomogenous species was not discussed, but in the case of *R. parvisporum* the mycelium encircled and spread over the scales.

R. parvisporum differs from the species on mites in several respects, such as the shorter, non-septate character of the conidiophores, their regular inflation (up to 2 μ) and the smaller conidia.

[2] This species has never been available for study and comparison, and therefore has not been accepted as the type species. (See The Genera of Fungi (Clements and Shear), p. 389. 1931).

In culture, according to Petch, this species frequently produces conidiophores in groups on the mycelium so that the fungus might be taken for a *Cladobotryum* with decumbent conidiophores.

The second entomogenous species, *Rhinotrichum album,* was described by Petch (**3**) in 1926 on *Lecanium hemisphaericum.* In 1931 (**4**, p. 61) he stated that it was synonymous with *Gonatorrhodiella coccorum* described by him in 1925 (**2**, pp. 178–181). In discussing this fungus Petch questioned its proper allocation to the genus *Gonatorrhodiella* calling attention to the manner of growth as follows: "The growth of the conidiophores is definite. It terminates in a definite, apical segment and does not subsequently grow through that segment," adding that "the apical segment is formed before the swellings on the conidiophore." In reference to the synonymy of *R. album* and *G. coccorum* (**4**, p. 61) Petch stated that "further collections of this fungus (*R. album*) and their culture on Quaker Oat agar have shown that it is the same as *G. coccorum* Petch." While the synonymy of these two species is announced there is no formal combination of the name or an emended or additional description of the fungus. The original descriptions of the two species *G. coccorum* and *R. album* differ in several characters. In *G. coccorum* the conidiophores are 0.2 mm. high, in *R. album* 0.1 mm., in *G. coccorum* they are described as inflated below each septum, while in *R. album* they are described as equal and slightly broader and clavate at the apex.

In the early description of *G. coccorum* mention is made of a whorled arrangement of the sterigmata below the septa as well as a scattered arrangement over the terminal segment and along the conidiophore. In addition the conidia are in chains, a character not mentioned in the description of *R. album* or of any other species of *Rhinotrichum.* A slight difference is also noticed in the size of the conidia in the respective descriptions of these two species. It is quite possible that these differences may be due to the age and condition of the material. However, in view of these discrepancies and in the absence of an emended description, the lack of material for comparison, and the occurrence of the fungus on an unrelated host the writer hesitates to allocate the fungus on mites to *R. album.* Therefore it is thought best to describe the

fungus on the mites as new even though later it may be shown to be Petch's species. Accordingly, the fungus is designated *R. depauperatum* and described as follows:

Rhinotrichum depauperatum sp. nov.

Effuse, cobwebby, white to pale gray in age; sterile hyphae repent or suberect; fertile hyphae long, 75–200 μ or longer, 1.5–2 μ in diam., ascending, flexuous, closely septate, sometimes slightly enlarged below the septum, terminal cell ovate-conoid and sporiferous; conidia borne on sterigmata along entire length of the conidiophore, sterigmata 1–2 μ in length, conidia ovoid, 1.5–2 $\times$ 2.5–3 μ, smooth, hyaline.

Late effusum arachnoideum, albo-griseum; hyphis sterilibus repentibus vel ascendentibus; hyphis fertilibus perlongis, 75–200 μ vel longioribus, 1.5–2 μ diam., ascendentibus, flexuosis, breve septatis, interdum infra septa leniter inflatis, cellula terminali ovoideo-conoidea et sporigena; conidiis in sterigmatibus 1–2 μ longis per longitudinem totam conidiophori, ovoideis, 1.5–2 $\times$ 2.5–3 μ, levibus, hyalinis.

On *Paratetranychus yothersi* (McGregor) on leaves of *Piaropus crassipes* (Mart.) Britton. Type collected at Maitland, Florida, Jan. 1939 and deposited in the Mycological Collections of the Bureau of Plant Industry (No. *72565*). A portion of the type collection deposited in the herbarium of the Florida Agr. Exp. Sta., Gainesville.

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WASHINGTON, D. C.

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MYCOLOGICAL NOTES. IV

C. L. Shear

16. Sphaeronaema Fries

Various interpretations of the characters and limitations of this genus have been made since it was first described by Fries as *Sphaeronema* Obs. Myc. **1**: 187. 1815. The question may be raised as to whether the name should be spelled *Sphaeronema* as Fries originally used it or *Sphaeronaema* as changed by him in Syst. Myc. **2**: 535. 1823, and Summa Veg. Scand. 400. 1849. The change was made, he says, because the Greek *nema* means thread and does not seem as appropriate as *naema* referring to the out-flowing of the spore globule. Saccardo and recent authors follow the later form. Sprengel likewise considering *nema* inappropriate also suggested a new name for the genus, *Sphaeromyxa* in Linn. Syst. Veg. Ed. 16, **4**: 406. 1827, and transferred all of Fries' species. Fries described the genus *Sphaeronema* as having "perithecia" of various shapes, frequently stalk-like or cylindrical with spores forming a gelatinous globule at the apex. Ten species, most of which had previously been referred to *Sphaeria,* were included and two were illustrated, *S. cladonisca* Obs. Myc. *pl. 2, f. 7* showing various forms, and *S. ventricosa* l.c., *pl. 2, f. 8.* Fries again in Syst. Myc. **2**: 535. 1823, described it in the same way and included 15 species, the first being *S. subulatum.* His next treatment was in Summa Veg. Scand. 400. 1849. There he lists 12 species, dividing them into three subgenera on the basis of the character of the pycnidial wall. (*a*) *Zythinia.* Pycnidia soft waxy, including *S. subulatum, S. rufum* and *S. aciculare.* (*b*) *Melanocybe.* Pycnidia carbonaceous and spore globule black. (*c*) *Genuina.* Pycnidia horny carbonaceous and spore globule white. The first species in the last group, which from the name he evidently intended to be regarded as typical, is *S. cladoniscum,* the second *S. cylindricum.* In the meantime Corda Icon. Fung. **1**: 25. 1837; **3**: 27. 1840; **4**: 39. 1840; and

5: 31. 1842 had used the name and added several new species. The first species he described was *S. vitreum* l.c. **1**: *f. 297.* He modified Fries' description somewhat, restricting its application to forms with membranaceous or coriaceous-fleshy pycnidia with more or less elongate necks and simple spores. Corda appears to have been the first to give a description and illustration of spores of the genus. He also says very emphatically "asci nulli!"

During the period between Corda and Saccardo very little was added to the knowledge of this group. Until the introduction of the type method of fixing, in part at least, the application of generic and specific names only general macroscopic characters were used in separating these groups. As microscopic characters were studied and described, greater importance was placed on the character of the tissues of pycnidial walls, and the spores and sporophores. Saccardo Syll. Fung. **3**: 185. 1884, accepted the genus as originally described by Fries, except that he restricted it to species having hyaline or sub-hyaline, simple spores and included 83 species. In 1896 Jaczewski published a monograph of the genus, Monographie du genre *Sphaeronema* Fries. His concept was similar to that of Saccardo, but much broader. He did not designate a type, but after discussing the various descriptions of Fries, Montagne, Karsten and Saccardo, gave a revised description which translated is as follows:

"Pycnidia membranaceous, coriaceous or soft fleshy, black carbonaceous or colored, innate or superficial, cylindrical, pyriform, or globose with emerged beak or ostioles, spores hyaline or subhyaline, rarely brown, simple or plurilocular." He included 72 species which, according to some recent authors, includes members of various widely separated genera.

Von Höhnel in 1917, Hedwigia **59**: 273, undertook a revision of this genus, fixing its application by the type method. He selected as type the first species in Fries' list in Syst. Myc. **2**: 535. 1823, which was *S. subulatum* (Tode) Fries. Having chosen this species it was necessary to determine exactly what plant this name really belonged to. This problem was not so easy to solve. Tode, Fungi Meckl. **2**: 44. *pl. 15, f. 117.* 1791, described and illustrated *Sphaeria subulata,* but just what Tode had can not be determined

with certainty at present, as none of his specimens are extant, and his description might apply to either a pycnidial or a perithecial form. From his statement that the spore globule which forms at the ostiole becomes black, it appears that he probably had a *Melanospora* and not one of the group of fungi to which the name *Sphaeronaema* has been applied by Fries and later authors. The question then arises what was the plant to which Fries applied the name *subulata?* This species is No. 6 in his first list, Obs. Myc. l.c., without description. He cites *Sphaeria subulata* Pers. and also Tode as synonyms. In Syst. Myc. l.c., he cites Tode and Persoon and Albertini and Schweinitz, also Nees, Syst. Myc. *f. 345,* but most important of all for purposes of exact identification is his citation of his own Scleromyceti Sueciae *325.* The specimens of this number which we have examined were unfortunately destroyed by insects or show no fructifications of a *Sphaeronaema.* Von Höhnel, Frag. Myc. *32,* 1902, says he did not see Fries' specimen of this species and bases his identification of it on a specimen of Fuckel, Fungi Rhen. *773,* which was described by Fuckel as the type of a new genus of ascomycete, *Eleutheromyces.* Notwithstanding the fact that Fuckel described, illustrated and gave measurements of the asci, von Höhnel maintains that Fuckel's fungus is a pycnidial form identical with that described by Fries as *S. subulata* and that therefore *Sphaeronaema* Fries and *Eleutheromyces* of Fuckel are synonyms. This seems entirely unjustifiable. Unfortunately von Höhnel's statement was accepted in Clements and Shear, The Key to the Genera of Fungi 360, where *Eleutheromyces* as a synonym should be deleted and the type of *Sphaeronaema* changed to *S. cylindricum.* The question of the real nature of *Eleutheromyces* will be discussed later. Fortunately, Petrak and Sydow were able to examine a good original specimen of *Sphaeronaema subulatum* Fries from Fries' Herbarium which they describe, Ann. Myc. **21**: 361. 1923, as follows: Fructifications on the upper surface of the pileus and gills of an agaric, scattered, superficial, very variable in form, globular or oval, more or less elongated into a beak with round apex, 200–400 μ in diameter, wall of several layers of irregularly rounded hyaline cells; conidiophores covering the whole inner surface of

the wall; neck filiform and very variable in length, simple or somewhat branched scarcely a micron thick; conidia united in a slimy mass, elongate elliptical, 1-celled, somewhat inequilateral with an indistinct hyaline cilium about $6 \times 0.5\ \mu$ at the apex. At the base of the spore there is a portion of the sporophore attached about 1.5–2.5 μ long. This is the fungus which Grove (British Stem & Leaf Fungi **2**: 115. 1937.) describes and figures as *Sphaeronemella subulata* comb. nov., and A. L. Smith (Trans. Brit. Myc. Soc. **1**: 199. 1897.) as *Sphaeronemella oxyspora* (Berk.) Sacc. The fungus which von Höhnel found on one of Fuckel's specimens (No. *773*) is probably the same, but is not the fungus which Fuckel described and illustrated as *Eleutheromyces*. If *Sphaeronaema subulatum* Fries be accepted as the type of the genus, as von Höhnel maintains, it would restrict the application of the name to a very small group of species of the Nectrioidaceae and change almost entirely the current usage of the name as applied by Saccardo and recent authors.

Petrak and Sydow l.c., in discussing *Sphaeronaema*, maintain with much more reason that *S. cladoniscum* or *S. cylindricum* which are the first two species mentioned and illustrated by Fries, Obs. Myc. l.c., and are also the first species under Fries' section *Genuina* in Summa Veg. Scand. 400. 1849, should be taken as the type. These two names are regarded as synonyms by von Höhnel and recent authors. This would make *Sphaeronemina* von Höhnel, l.c. p. 274, a synonym of *Sphaeronaema*, both having the same type, *S. cylindricum* (Tode) Fries.

More information regarding the taxonomic value of the various criteria which have been used in segregating genera in this group and also regarding their life cycles must be obtained before any great improvement can be made in their classification. Von Höhnel states that a dozen or more genera are represented by the species included by Saccardo. Some of these new genera which he has proposed, however, are of doubtful value.

17. SPHAERONAEMELLA Karst.

This genus was described in Hedwigia **33**: 17. 1884, with the monotype *S. Helvellae* Karst., specimens of which were distributed

by the author in his Fungi Fenniae Exsiccati as no. *674*. Jaczewski in his monograph of *Sphaeronema*, p. 303, 1896, states that he examined Karsten's type and that the pycnidia have long, cylindrical, spirally twisted, penicillate beaks and spores $9 \times 4.5\ \mu$. No sporophores are mentioned. Later Diedicke, Krypt.-Fl. Brand. IX. **4**: 694. 1914, states that the original specimen of Karsten's fungus which he examined showed the pycnidium to be parenchymatic and the beak composed of long fascicled hyphae, sporophores lacking, spores simple, elliptical $7\text{–}13 \times 4\text{–}6\ \mu$. Von Höhnel discusses this genus in Hedwigia **60**: 151–155. 1918, and states that he had not seen the original specimens of Karsten's fungus, but that according to the accounts of Jaczewski and Diedicke as cited, since no sporophores were found, he has no doubt that Karsten's fungus is an ascomycete very similar to *Ceratostomella* in which the asci have disappeared, but belonging to the Hypocreaceae. For a fungus similar in appearance to Karsten's, but which he regards as a pycnidial form, he establishes a new genus, *Hyalopycnis*, with the type *H. hyalina* von Höhnel, and adds two other species, *H. vitrea* (Corda) von Höhnel and *Sphaeronaema blepharistoma* Berk., Mag. Zoöl. & Bot. **1**: 512. 1837. He characterizes the genus as having superficial, transparent pycnidia provided with thick beaks with fimbriate ostioles, and branched sporophores with single, elongate-cylindrical, 1-celled, hyaline conidia $6\text{–}14 \times 2.6\text{–}3.5\ \mu$. The character which he regards as most important and which he says separates the type from the species which are usually placed in *Sphaeronaemella*, is the plectenchymatic structure of the pycnidium, and the closely parallel, hyaline, thin-walled, sparingly septate hyphae in the beak. From the information thus far available and until much more evidence is produced, it does not seem that von Höhnel is justified in treating Karsten's fungus as an ascogenous genus, and therefore we consider his *Hyalopycnis* as a synonym of *Sphaeronaemella* Karst.

Grove, British Stem and Leaf Fungi **2**: 115. 1937, has emended the description to include spores having appendages and has transferred *Sphaeronaema subulatum* Fries to *Sphaeronaemella*, making the new combination *S. subulata* (Fries) Grove and including *Sphaeronaema oxysporum* Berk. as a synonym. We are

inclined to accept this treatment of the genus until more satisfactory information is obtained in regard to the structure and life cycles of the species involved. If Grove's concept of this genus be accepted it follows as intimated in his note (l.c. p. 116) that *Eleutheromycella* von Höhnel, Frag. Myc. *178,* 1908, becomes a synonym of *Sphaeronaemella.* The type of von Höhnel's genus, *E. mycophila,* apparently differs from *Sphaeronaemella subulata* (Fries) Grove in having a conical papilla instead of a beak on the pycnidium, and in longer spores and setae.

18. ELEUTHEROMYCES Fuckel

This genus was described by Fuckel, Symb. Myc. 183. *pl. 4, f. 52.* 1869. It was based on a single species which he called *E. subulatus* Fuckel. He cites *Sphaeronaema subulatum* (Tode) Fries, Syst. Myc. **2**: 536, as a synonym and his own specimen No. *773* in Fungi Rhenani Exsiccati on a decaying agaric as representing the species. As already stated above under note 16, von Höhnel examined a specimen of this number, and finding only free spores concluded that Fuckel was mistaken in his description and did not have an ascomycete, in spite of the fact that he described and illustrated asci and ascospores and gave measurements of the asci ($52 \times 2.5\ \mu$). That there is an ascomycete on decaying agarics corresponding to Fuckel's description and illustration seems proven by the evidence of later mycologists. Winter (Die Pilze **2**: 93. 1887.) reports the species on *Polyporus betulinus* and gives the following measurements: Asci $48–52 \times 2.5–3\ \mu$, ascospores $4–6 \times 1.5\ \mu$. His illustration, however, on page 84 is evidently copied from Fuckel.

Petch (Jour. Bot. **73**: 186. 1935.) pointed out von Höhnel's error in assuming that Fuckel's fungus was not an ascomycete, and cited Sacc. Syll. Fung. **17**: 779. 1905, and **22**: 1142, 1913, where Saccardo states that there is an ascogenous fungus agreeing with Fuckel's description. Saccardo (Michelia **1**: 50. 1879.) also describes a specimen which he collected in Italy in 1876 which had asci $50 \times 4\ \mu$ and ascospores $5–6 \times 1.75–2.5\ \mu$ with setae at each end. Saccardo also states that there is a pycnidial fungus very similar in appearance to *Eleutheromyces* which may be part of its life cycle. The best illustration of the ascogenous form of this

fungus is found in Ellis and Everhart, N. Am. Pyren. *pl. 14, f. 6–12.* 1892. It is possible, however, that this may represent a different, but closely related species, as the asci and ascospores do not agree well with Fuckel's illustration. From the evidence submitted above it seems rather conclusive that von Höhnel's statement that Fuckel's fungus is a pycnidial form which he regards as the *Sphaeronaema subulatum* of Fries and which he takes as the type of the genus *Sphaeronaema* is a mistake.

19. SPHAERIA PUGILLUS Schw.

This fungus was described by Schweinitz in his Syn. Fung. Car. 38. 1822, as follows:

107. *Pugillus* Sz.

S. circinata majuscula immersa atra, ostiolis in cylindrum compressum conflatis, supra divergentibus, singulis capitulo lutescente coronatis.

Ad ligna dura acerina cortice orbata crescens, non male refert pugillum clausum. Profunde in substantiam ligni immersae sphaerulae sunt circinantes aterrimae, numerosae, circumdatae materia alba, e ligno orta. Ostiola connata in cylindrum compressum, interdum oblongo-ellipticum ascendunt per lignum et ad semilinearam altitudinem supra lignum. Tum cylindrus dividitur in 3, 4, 5 ostiola divergentia, ut digiti pugilli subclausi. Horum singulum est forma cylindrica, transversim plicatum, aterrimum, coronatum capitulo, sphaeriae forma, lutescente, viridi pruinato, pupilla nigra.

Fries in Syst. Myc. **2**: sec. 2, p. 383. 1823, also describes this species as follows:

135. *S. Pugillus,* ligno immersa, superne conceptaculo tecta, stromate albido, ostiolis in cylindrum compressum conflatis, supra divergentibus.

Schwein.! l.c. n. 107.

Priori affinis, stromate subdisco lutescente. In ligno nidulant perithecia circinantia, numerosa (minus conferta, quam in priori) stromate ligneo cincta. Cylinder erumpens (conceptatulum) dividitur in ostiola 3–5 divergentia. "quorum singulum est forma cylindrica, transversim plicatum, aterrimum, coronatum capitulo, Sphaeriae forma, lutescente, viridipruinato, papilla nigra" Schwein. l.c. Ad ligna duriora acerina cortice orbata in Carolina. (v.s.)

Fries' description was evidently based on a part of the original specimen described by Schweinitz.

In 1832 Schweinitz in his Syn. N. Am. Fung. 200, No. *1322,* lists the species as follows:

1322. 175. S. Pugillus, L. v. S., Syn. Car. 107, F. 135, in putrido ligno etiam Pennsylvania obvia, quanquam rara.

The next mention we find of this species is by Curtis, Geol. & Nat. Hist. Surv. N. Car. III, Bot., p. 142. 1867, where he lists it as *Valsa pugillus*. In 1878 W. C. Stevenson, Jr., published an article, entitled "Additions to Mr. Cooke's paper on the Valsei of the United States," Proc. Acad. Nat. Sci., Phil. **30**: 86–88. 1878, in which he has the following note on a specimen of this species which he found at the Philadelphia Academy:

"97. *Valsa pugillus*, Schw. Am. Bor. *1322*.
Evidently a *Sphaeronema*."

As noted by Schweinitz, Syn. N. Am. Fun. l.c., he had two specimens which he referred to this species, the original from Salem, North Carolina, and the other from Pennsylvania. The specimen referred to by Stevenson was probably that from Pennsylvania, which is found in the mounted collection of Schweinitz' fungi at the Philadelphia Academy. The next mention of it we find is by Cooke, Grevillea **13**: 38. 1884. He refers this species to *Sphaeronema*, simply quoting Stevenson, l.c. Ellis and Everhart in N. Am. Pyren. No. 470, 1892, also cite Stevenson's note.

There is no evidence that either Cooke or Ellis examined an authentic specimen. As we have pointed out before, Schweinitz' habit of including specimens from Pennsylvania or other localities in the same packet with the original material from North Carolina has led to much confusion in the interpretation of his species and this probably accounts for the fact that Stevenson found a "*Sphaeronema*" in Schweinitz' specimen. We have not examined the specimen in the mounted collection.

Fortunately there is still preserved in Schweinitz' original autographed packet a small specimen which agrees perfectly with his original description as quoted above and also that given by Fries, which was evidently from part of the same specimen. It bears a portion of a gummed paper strip which indicates that it was from his early material which he had first mounted on sheets with gummed strips. There is also a bit of this same specimen in the Michener herbarium in the Bureau of Plant Industry Collections. We have made four gatherings of specimens which agree with this

species and from a study of these and the original specimen referred to we find they clearly belong to the genus *Camarops,* as we have interpreted and discussed it in our Mycological Notes II, Mycologia **30**: 586. 1938. The stromata are entirely buried in the much decayed wood of old logs of deciduous trees. The ostioles are united in a more or less erumpent disk which, when fully developed, shows the characteristic appearance suggested by Schweinitz' specific name *pugillus.* The wood in which the perithecia are embedded is usually a pale yellowish or saffron color. The perithecia are crowded and membranaceous or somewhat coriaceous and subglobose to flask shaped, irregular and beaked. The beaks vary greatly in length. In many cases they do not project above the surface of the stroma. The ascospores are pale yellowish, elliptical, 5–6 × 2.4–3 μ. They have the thin hyaline envelope characteristic of the other species of this genus. It will be noted that the spores are almost identical in size, shape and general character with those of *C. lutea* (Alb. & Schw.) Shear and it is possible that this may be a variety of that species, though in the abundant material we have collected we have found no stromata which quite match the smallest in the material illustrated in our figure 1, l.c. We propose for Schweinitz' plant the name **Camarops pugillus** (Schw.) Shear comb. nov. Besides the type we have seen only the following specimens which we have gathered:

No. *5593,* on decaying log of maple or *Liriodendron,* Arlington Cemetery, Va., Mar. 1927.
No. *4238* on beech log, Arlington Co., Va., Oct. 22, 1933.
No. *4237* on *Liriodendron,* Arlington Cemetery, Va., Feb. 1939.
No. *4239* on *Rhododendron,* Indian Gap, Tenn., Aug. 1939.

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STUDIES ON CARYOSPORA PUTAMINUM [1]

WALTER F. JEFFERS

(WITH 2 FIGURES)

INTRODUCTION

Caryospora putaminum is an Ascomycete belonging to the family Sphaeriaceae. It is of world-wide occurrence, having been reported in North and Central America, China, France, Germany, India, Italy, and Japan. This organism appears to be strictly saprophytic, occurring mainly on old peach stones. It has also been found on acorns, hickory nuts, seeds of "*Buckaniana latifolia*," *Prunus cerasus*, and *Prunus domestica*, and on old wood of *Maclura aurantiaca*. The author has collected it recently at Berwyn, Md., on a piece of coconut (*Cocos nucifera*) shell.

Caryospora putaminum has been described many times, but with the exception of the gross description of perithecia, asci, and ascospores, no detailed study has been made. As this fungus has large perithecia and ascospores, it was considered a good subject for cytological, anatomical, and cultural studies.

During these studies on *C. putaminum* another form of this genus was found which differed from all previously described species. A complete description of this form is given, and it is described as a new species.

CULTURAL STUDIES

In order to determine the best conditions for perithecial production, various culture media were tested under different environmental conditions. Best growth occurred on peach-stone extract agar and on pea-extract agar. Good growth also occurred on regular malt-extract agar. The peach-stone agar was made by boiling peach stones containing perithecia of *Caryospora putaminum* and adding this extract to the ingredients of malt-extract agar.

[1] Scientific Contribution No. 501, Department of Botany, University of Maryland Agricultural Experiment Station.

The pea agar was made by adding the juice from fresh or canned peas to malt-extract agar.

On peach-stone agar young cultures were olive-grey in color; as they matured the aerial mycelium generally turned dark brown, while the substrate became black. When cultures were grown on rather concentrated pea extract agar, juice from 400 gms. of fresh peas per liter of agar, the mycelium varied from light grey to white in color. The aerial mycelium formed a dense layer, which became very wrinkled and sometimes cracked. Perithecia did not occur on this medium, but spermagonia and sclerotia were sometimes produced. When the pea extract was about half as concentrated as above or when juice from canned peas was used, the mycelium was light brown in color, and most cultures produced spermagonia in abundance. Perithecia occasionally formed on this less concentrated medium.

On peach-stone agar perithecia were produced over a pH range of 4.7 to 6.7; best production occurred between 5.0 and 6.0. On pea-extract agar spermagonia were produced over a pH range of 4.2 to 6.5; best production occurred between 4.7 and 6.0. The maximum and minimum pH values at which perithecia and spermagonia were produced varied somewhat in different isolants.

The optimum temperature range for growth of *Caryospora putaminum* is about 25–28° C. The maximum growth limit is close to 35° C., while the minimum temperature at which growth will occur is below 5° C.

Good growth and production of perithecia occurred when cultures were stored in semi or total darkness; but when cultures were placed in full sunlight, growth was somewhat decreased and only a few perithecia were formed.

Attempts were made to determine if there were any differences in cultures from 2, 3, and 4-spored asci. In one experiment whole asci containing these different numbers of spores were isolated, and in another test single spore isolations were made of each spore from 2, 3, 4, and 5-spored asci. In each instance there was no difference in the type of growth, and all of the resulting perithecia contained asci with various numbers of spores; as usual, 3-spored asci predominated.

ANATOMICAL AND CYTOLOGICAL STUDIES

Perithecia: Typical perithecia are 0.5–1.2 mm. in diameter and usually about the same in height. They are black in color and carbonaceous in texture. Naturally produced perithecia are usually slightly longer than broad, but in culture this ratio may be reversed. Mature perithecia are conical in shape and converge abruptly to a narrow tip containing a circular ostiole. The ostiole is about 0.1 mm. in diameter. The perithecial wall is usually about 125 μ thick, being composed of thick-walled cells which make up definite pseudoparenchymatous tissue. These cells are roughly rectangular in shape, average about $8 \times 6\,\mu$, and are uninucleate. Perithecia which occur on peach stones have no basal parenchyma, the sides being attached directly to the substrate. This may also be true of perithecia in culture, or the wall may completely enclose the perithecial contents. On peach stones the perithecia are entirely superficial, but in culture they may be partly sunken. A peach pit containing perithecia of *Caryospora putaminum* is shown in figure 1, *A*.

Perithecia occur both inside and outside intact peach stones. Sometimes so many perithecia are inside that the entire cavity becomes filled with a mass of ascospores. At other times perithecia are only on the inside, and sometimes only on the outside of the stone. Pits which are so firm that they must be forcibly opened often contain perithecia. Their origin is possibly due to mycelial strands which enter small openings at the union of the two halves of the stone or which travel through the canaliculi of the stone. Small compact perithecia have been found within these canals.

Nearly mature perithecia contain a dense mass of paraphyses and asci. Apparently there is a gelatinous secretion present, for the perithecial content is jellylike and expands and contracts according to the moisture conditions. When mature perithecia become moist, the contents swell and ascospores are exuded through the ostiole as a sticky mass.

Collections of perithecia were made during each month of the year to study the seasonal development of asci and ascospores. Asci and spores in different stages of development were always

found. In natural material most spores mature during warm weather, spore discharge taking place during wet periods. Isolations have frequently been made from spores collected during the winter months; and as these spores gave good germination, it appears that this is the main method of overwintering. Ascospores are able to withstand periods of dryness, for stones kept in the laboratory for 2 months still contained spores which gave 100 per cent germination.

In culture the first definite evidence of a perithecium is a small knot of hyphae about 20–40 μ in diameter. Soon an outer wall of pseudoparenchymatous tissue becomes evident. This wall surrounds a mass of thin-walled, densely cytoplasmic cells which appear to be uninucleate. When the perithecium is about 1½ months old, larger cells become visible in the center of the perithecium. These cells appear to be the primary ascogenous hyphae, for soon a fertile layer develops in the base of the perithecium. From this layer of ascogenous hyphae the young asci arise and grow up through the thick mass of paraphyses which had previously formed.

Asci and ascospores: Caryospora putaminum has not been observed to produce eight-spored asci. The most frequent number of spores per ascus is three; next in abundance are 4, 2, 5, 1, 6, and 7 in the order listed. These figures hold true for artificially and naturally produced material. Table I summarizes the occurrence of asci containing various numbers of spores in naturally and artificially produced perithecia.

TABLE I

OCCURRENCE OF ASCI CONTAINING VARIOUS NUMBERS OF SPORES IN NATURALLY AND ARTIFICIALLY PRODUCED PERITHECIA

Number of spores per ascus	Asci produced	
	Naturally	Artificially
1	6	11
2	85	75
3	222	180
4	153	86
5	10	4
6	6	1
7	2	0
8	0	0

Asci of this organism are large, some attaining a length of 300 μ

and a width of 75 μ. The ascus wall is very thin and evanescent, usually being rather difficult to see. The ascus is club-shaped, rounded at the top, and tapers to a rather slender stalk at the base. Asci and ascospores are shown in figure 1, *B* and *C*.

Asci originate either from normal crozier development or as a swelling of a branch of the ascogenous hyphae without crozier formation. The ascogenous hyphae are so branched that they are very difficult to follow in sectioned material. Smear mounts show that the branching is irregular and very profuse.

Normal croziers have been observed in which fusion had just occurred in the penultimate cell while the ultimate and antipenultimate cells, each containing one nucleus, were about to fuse. Fusion nuclei vary from 3.5 to 6.0 μ while accompanying nucleoli range from 1.5 to 2.6 μ. Asci remain in the uninucleate condition until they are quite large. The general size of asci containing the fusion nucleus is $34 \times 9\ \mu$ to $85 \times 20\ \mu$. This is by far the commonest stage in ascus development to be observed. As the fusion nucleus prepares to divide, the nucleolus disappears and a number of small, round chromatin bodies become evident. These ball-like particles are connected to one another by a network of chromatic strands. Usually there are ten or more such particles per nucleus. Other stages in the division of the fusion nucleus were observed, but no chromosome counts were obtained. To determine the possibility of a daily periodicity in the nuclear divisions in the asci, material was fixed at various times during the day and night. Only a few nuclear divisions were observed in any of this material. A few binucleate asci were noted and in one instance a young 3-spored ascus was found which contained one nucleus per spore while five nuclei remained in the epiplasm. Frequently asci were noted with nuclei in the epiplasm; the spores of such asci contained one nucleus in each of their two main cells. It seems likely that each spore contains one nucleus which divides to form a binucleate spore. This was found to be true in a young 3-spored ascus where the median spore septations had not yet formed; here there was one nucleus in each spore and two of these nuclei appeared to be in an early stage of division. Soon after the spores become binucleate a median septum is formed so that each cell contains one nucleus. Each ascospore is surrounded by a distinct hyaline envelope.

Asci containing one or more abortive spores have been noted. Nuclei could not be seen in such spores. Nuclei which are not originally included in a spore are left in the epiplasm and finally disintegrate.

Ascospores are delimited, as usual in the Ascomycetes, by the process of free cell formation. Young asci contain much dense cytoplasm but before spores are delimited they become quite vacuolate. Soon the outer spore walls and the median septum appear; these are followed by the terminal septations. Each spore contains one distinct median septum and from one to seven septa in each of the narrow tips. Only the two main cells contain nuclei. These nuclei average about 3.3 μ in diameter while the nucleoli average about 1.5 μ. The spores at this time are hyaline but soon a brown pigment begins to appear in the form of circular spots over the external walls. These spots merge and finally the mature ascospores are solid black in color. Mature spores are brittle in texture.

Measurements of several hundred ascospores showed an average size of 97 $\times$ 45 μ. The largest spore so far noted was 139.1 $\times$ 65.6 μ. As the number of spores per ascus increased, the spore size decreased; this is shown in the following measurements:

Spores from	Average size of spore, μ
2-spored asci	106×50
3-spored asci	92×42
4-spored asci	84×40
5-spored asci	72×34

Most spores were slightly more than twice as long as wide. Occasionally spores occurred which were very abnormal in shape, some being very slender while others were almost spherical.

Ascospores germinate in two to three days when placed on a favorable medium. Two germ tubes are produced, one from each tip. Often the hyaline envelope which surrounds each spore prevents the immediate exit of the germ tube. In such instances the tube bends back and grows between the spore wall and the envelope. Many branches arise, and finally the envelope is ruptured in such a manner that the spore appears to have many germ pores. Germ tubes are 4–6 μ in diameter at the base and taper to 1–2 μ at the tip. Many branches are formed, and soon each spore is

surrounded by radiating strands of mycelium. Many anastomoses of various types occur in the young mycelium. Most mycelium is uninucleate, but multinucleate hyphae also occur.

The aerial mycelium of *Caryospora putaminum* is brown in color, sparsely septate, and varies from 1.0 to 3.8 μ in diameter. Old hyphae possess knoblike thickenings over the outer surface. These thickenings form gradually, and various stages in their formation are usually noticeable. Nutritive mycelium lacks the wall thickenings and usually is more irregular in size. The usual diameter is from 1.5 to 5.0 μ, but swellings frequently occur which attain a diameter of 15 μ.

Paraphyses are very long and slender, some being 500 μ in length; their width varies from 1 to 3.5 μ. They are septate, each cell being uninucleate. Tips of paraphyses are pointed. A number of division figures have been seen in paraphyses, but due to their small size it was difficult to make any detailed study. The chromosomes appear to be spherical but lie so close together that they usually appear as a constricted rod-shaped body. At anaphase there are apparently two groups, each containing three or four of the spherical chromosomes.

Spermagonia and spermatia: When cultured on pea-extract agar, *Caryospora putaminum* forms small black carbonaceous spermagonia (FIG. 1, *D*). These are from 150 to 300 μ in height and from 120 to 180 μ wide. When mature, a circular ostiole about 30 μ in diameter is present in the top. The walls of the spermagonium are about 40 μ in thickness and composed of a pseudoparenchymatous tissue. Spermatia are exuded through the ostiole as a white cohering mass; they usually form a white ball-like body on the spermagonium.

The spermatia are produced from short sac-like stalks which line the interior of the spermagonium. Spermatia are single-celled, hyaline, and measure about 2.8×1.9 μ. Examination of unstained spermatia shows one or two small circular bodies in each cell. These same bodies are evident when spermatia are stained in aceto-carmine. When fixed in chromo-acetic acid or Flemming's medium, the spermatia do not show these round central bodies but do show dark crescent-shaped bodies which usually lie against the wall. These curved bodies are evidently chromatic in nature but

do not have the appearance of the other nuclei observed in this organism.

Transfers from the masses of spermatia on the spermagonia gave normal cultures of *Caryospora,* but since it was possible that a strand of mycelium was also transferred, hanging-drop cultures of spermatia were made. Drops of various agars were placed on sterile cover slips, inoculated with spermatia and sealed to sterile depression slides. These slides were examined daily for 10 days, but no evidence of germination by the spermatia was noted. Conditions for growth were favorable, for strands of mycelium which were transferred at the same time made good growth.

Most evidence indicates that spermatia are not absolutely necessary for sexual reproduction of *Caryospora putaminum* and are not concerned in dissemination. Points in favor of this view are as follows:

1. Spermagonia and spermatia were not observed to occur in nature.
2. No cytological evidence of spermatia acting as fertilizing agents could be found.
3. Most cultures did not form spermagonia and perithecia on the same medium.
4. When spermagonia and perithecia occurred in the same culture, the spermatia were not discharged until the perithecia were nearly mature.
5. Spermatia were not observed to germinate.

Apparently the initiators necessary for formation of perithecia and spermagonia are very similar, for cultures producing most perithecia on malt agars also form most spermagonia on pea-extract agar. Also when spermagonia and perithecia do occur in the same culture, the two structures may fuse to form a compound fruiting body. Spermagonia have been noted to occur as sunken cavities in the upper part of perithecia.

Sclerotia: Occasionally, on pea-extract agar, small white structures arose directly from the agar surface. These masses were irregular in shape; some were spherical, while others formed a thin layer. Upon examination, this material was found to consist of irregular-shaped cells which formed a sclerotium-like tissue. Transfer of this tissue gave normal cultures of *Caryospora.* No

similar material was observed to occur in nature, and in culture it is of rare occurrence.

TAXONOMIC STUDIES

The differentiating characters used in the description of the various species of *Caryospora* were obtained from the following sources: Arnaud (**1**), Currey (**2**), Ellis and Everhart (**3**) and (**4**), Fairman (**5**) and (**6**), Fries (**7**), Lindau (**9**), De Notaris (**10**), Peck (**12**), Saccardo (**14**), (**15**), and (**17**), Schweinitz (**17**), and Thumen (**19**). Macroscopic observations were made of all the following species except *Caryospora coffeae*. These specimens were examined either at the United States Department of Agriculture mycological herbarium or at the mycological herbarium of the New York City Botanical Garden. The following species of *Caryospora* have been described:

1. C. CALLICARPA (Curr.) Njtschke & Fuckel.

Perithecia large, ¾–1 mm., subglobose, papillate and ostiolate. Asci ovate-clavate, 210–260 × 60–70 μ, eight-spored. Spores large, 87–108 × 30–47 μ, uniseptate with one or two small loculi at each end. Occurs in North America on dead oak wood and on bark of *Populus nigra*.

2. C. CARIOSA Fairman.

Perithecia large, conic, black, ostiolate, mainly superficial. Asci oblong-cylindrical, 150 × 20 μ, 2–8-spored. Spores 36–43 × 13–17 μ, occasionally with extra septa near the ends. Occurs in North America on beech wood.

3. C. COFFEAE Pat.

Perithecia subglobose, black 0.5–1 mm., ostiolate. Asci club-shaped, 200–250 × 45–60 μ, 8-spored. Spores 80 × 20 μ, first uniseptate then five septate. Occurs on twigs of coffee trees in Venezuela.

4. C. LANGLOISII Ellis & Ev.

Perithecia slightly sunken, nearly 1 mm. wide, brownish-black, distinct ostiole, papilliform. Asci 120–140 × 40–45 μ, 8-spored. Spores 1-septate, somewhat constricted, 35–45 × 16–20 μ. Occurs in Louisiana on old canes of *Arundinaria*.

5. C. LICHENOPSIS (Mass.) Sacc.

Perithecia ½ to ¾ mm. in height. Asci 150–170 × 15–20 μ, 8-spored. Spores about 8-septate, 34–36 × 12–14 μ. Occurs in Italy on bark of living *Cerasus*.

6. C. MINOR Peck.

Perithecia 350–500 μ in height, conic, and ostiolate. Asci subcylindrical, 150 × 20 μ, 4 to 8-spored. Spores contain one median septum and sometimes 3 to 5 pseudosepta, 45–50 × 12 μ. Occurs in North America on pericarp of hickory nuts.

7. C. NUCLEARIA Thüm.

Perithecia 1 mm., carbonaceous, broad, and ostiolate. Asci elongate, 8-spored. Sporidia elongate, somewhat constricted, 4-loculate. Occurs in Italy on old olive pits.

8. C. OLEARUM (Cast.) Sacc.

Perithecia ¾ mm. in height, conic, and ostiolate. Asci 110–140 × 12–15 μ, 8-spored. Spores 28–32 × 9–10.5 μ, 5 to 7 septate. Occurs in Italy on bark of *Olea europea*.

9. C. PUTAMINUM (Schw.) De Not.

Perithecia 0.5 to 1.2 mm. in height, black, carbonaceous, papillate, and ostiolate. Asci clavate, 200–350 × 50–75 μ, 2 to 8-spored. Spores 60–150 × 35–65 μ, median septate with 1 to 5 loculi at each tip. Occurs mainly on old peach stones.

While examining peach stones for *Caryospora putaminum*, another form of this genus was found which differed from any of the species described above. The perithecia are black, carbonaceous in texture, and ostiolate. Perithecia vary from 400 to 750 μ in height and are usually about as broad as they are high. Asci are cylindrical-clavate, 150–180 × 35–50 μ, and are always eight-spored. These spores are light brown in color, median septate and usually possess one small septum at each tip. The tips are somewhat rounded. The average size of these spores is 45 × 24 μ. Ellis and Everhart (**4**) mentioned the occurrence, on peach stones, of an organism which they believed to be an eight-spored

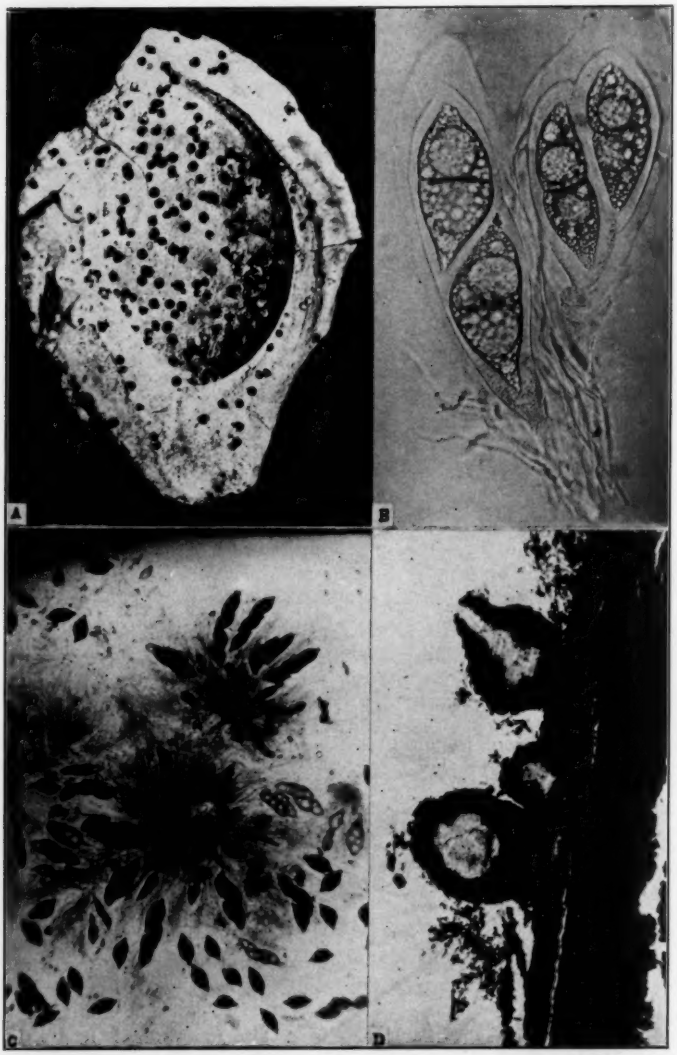

FIG. 1. *Caryospora putaminum*. *A*, perithecia on peach stone, × 2.5; *B*, two-spored asci, × 420; *C*, partial contents of a mature perithecium, × 90; *D*, spermagonia produced in culture, × 120.

form of *C. putaminum*. As they gave no further description and as this organism differs from the other known species of *Caryospora*, it is proposed as a new species.

Caryospora minima sp. nov.

Peritheciis sparsis, nigris, conicis, ostiolato, 400–700 μ altis; ascis clavato-cylindraceis, 150–180 μ longis × 35–50 μ crassis, 8-sporis; sporidiis 40–50 μ longis × 20–30 μ crassis, obtusus, uniseptatis, ad septum leniter constrictis, unoloculus ad extremitis, subfuscus. Habitat in putamine putrescente *Amygdalus persica* in Maryland.

Type collected at Berlin, Maryland, August 1938, and deposited in the Mycological Collections of the Bureau of Plant Industry (No. *71132*). Other collections were made at College Park and at Conway Station, Anne Arundel Co., Maryland. Portions of the type collection also deposited in the herbarium of the New York Botanical Garden, the Farlow herbarium of Harvard University, and the herbarium of the University of Michigan.

Single-spore isolations were made, and cultures were grown on various media. On peach-stone agar the mycelium is dark grey in color while the substrate is black. Perithecia are produced in culture but not very abundantly. The growth requirements of this fungus were almost identical with those of *C. putaminum*. Perithecia were produced over a pH range of 4.75 to 6.51. Pea-extract agar caused cultures of *C. minima* to become lighter in color, but neither spermagonia nor sclerotia were found.

Perithecia of *C. minima* are identical with those of *C. putaminum* except for the difference in size. The perithecial wall is made up of similar pseudoparenchymatous tissue while asci and paraphyses arise from a layer of hyphae in the base of the perithecium. During moist weather spores are exuded through the circular ostiole and form a compact mass on top of the perithecium.

Asci of *C. minima* are usually produced by crozier formation (FIG. 2, *A, B*). Occasionally asci arise directly from a branch of the ascogenous hyphae without crozier formation. Crozier formation is much more frequent in this species than in *C. putaminum*. The fusion nucleus in young asci of *C. minima* is about 3.8 μ in diameter with a dense nucleolus of 2.3 μ. The ascus elongates until it is about two-thirds full size; then the dense cytoplasm be-

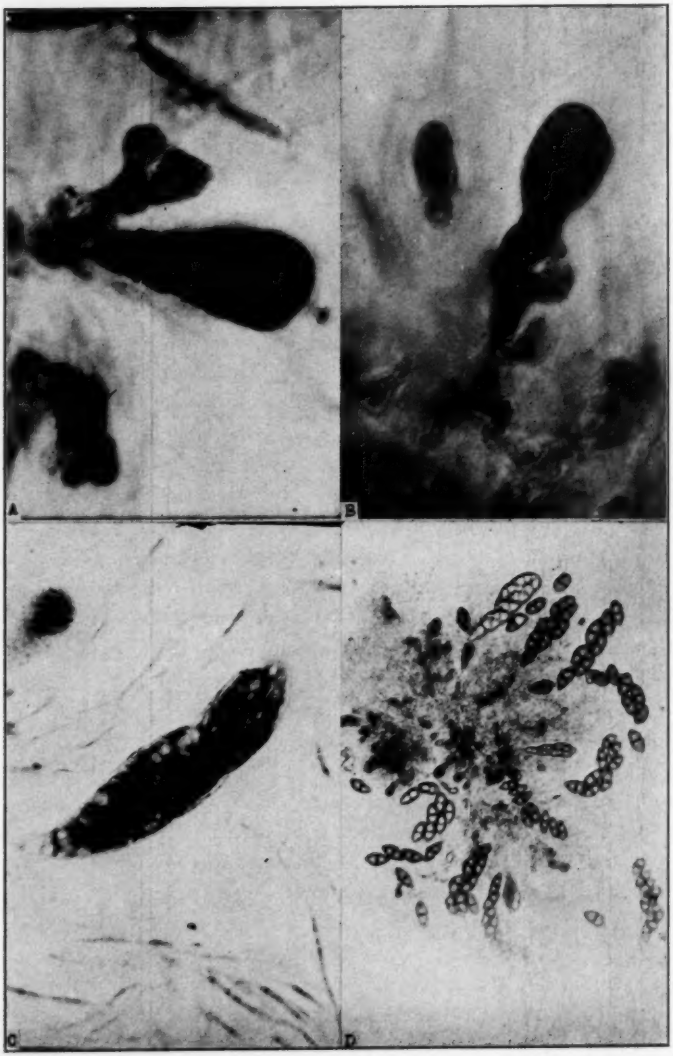

FIG. 2. *Caryospora minima*. *A*, early stage of crozier formation, × 2000; *B*, later stage of crozier formation, × 2000; *C*, ascus containing recently delimited ascospores, × 450; *D*, asci containing mature ascospores, × 150.

comes vacuolate and eight spores are delimited by the process of free cell formation (FIG. 2, *C*). Spores are at first hyaline and contain one median septum; each of the two cells thus formed contains one nucleus. As the spores mature, the color becomes light brown and a septum appears at each tip. Each spore is surrounded by a very thin hyaline layer which disappears upon drying. Asci and spores of *Caryospora minima* are shown in figure 2, *D*.

The mycelium of *Caryospora minima* is very similar to that of *Caryospora putaminum*, being brown in color, sparsely septate, and averaging about 2.5 μ in diameter. Most of the mycelial cells appear uninucleate, but the nuclear condition is often difficult to determine due to the many nuclear-like chromatin bodies which occur in the cells. Anastomoses occur frequently in young mycelium.

DISCUSSION

Caryospora putaminum differs from most Pyrenomycetes in that it forms various numbers of spores per ascus. Other fungi of this order form less than eight spores per ascus, but the number of spores usually remains constant. However, in *C. putaminum* the number of spores per ascus may range from one to seven, but an eight-spored ascus has not been observed.

Saccardo (**16**) made the only reference so far noted to the occurrence of spermagonia and spermatia in *Caryospora putaminum*. His description is as follows:

"Fungus spermogonium spermatiis minutissimis cylindraceis, oscillantibus." Saccardo's idea of movement by the spermatia was probably due to Brownian movement or surface tension phenomena, for no evidence of self-propulsion has been noted.

The only reference to the proposed *Caryospora minima* was made by Ellis and Everhart (**4**). They noted that such a fungus did exist but did not propose it as a separate species; their complete description is as follows:

"Occasionally a peach pit is met with, on which all the perithecia produce 8-spored asci, but in this case the sporidia are smaller, 30–50 $\times$ 18–20 μ. There are generally but two of the larger sporidia in an ascus."

Evidently they considered it as merely an eight-spored form of *Caryospora putaminum* and did not take into consideration the difference in perithecial size and spore shape. In *C. putaminum* perithecia always contain asci with varying numbers of spores per ascus, but in *C. minima* there are always eight spores per ascus.

Several workers doubt the validity of the genus *Caryospora*. Ellis and Everhart state that it is hardly more than a subgenus of *Trematosphaeria*. Peck (**12**) states that *Caryospora minima* may be a *Melanomma* or a *Trematosphaeria*. Höhnel (**8**) and others have noted the similarity between the genera *Sphaeria* and *Trematosphaeria*. The main source of confusion in the classification of *Caryospora* are the small septa which are usually found at the tip of each spore. The number of terminal septations per spore is variable and in some instances they are not even present. It seems that *Caryospora* constitutes a distinct genus but that its position in the Sphaeriaceae should be changed. Due to its terminal septations, *Caryospora* is now placed in the Phaeophragmiae, but as true cells are not formed by these septa, it seems likely that this genus should be included in the Phaeodidymae.

Tassi (**18**) reported the occurrence of an organism which he believed to be the pycnidial stage of *Caryospora putaminum*. He called this organism *Santiella putaminum* and described it as follows:

> Peritheciis sparsis, globoso-conicis, papillatis, subcarbonaceis, nigris, subsuperficialibus, basi insculptis, facile secedentibus, 200–250 μ diam., sporulis breve fusoideis, medio 1-septatis, intense fuligineis, non constrictis, crasse 2-guttalis, utrinque locello copuliformi V. obtusulo hyalino auctis 22–26 × 12.

He found this species in Italy on old stones of *Prunus domestica*. Tassi also reported another similar organism on old seeds of *Melia Azedarach;* this he called *Santiella oblonga*. Saccardo (**16**) also mentions these species, but he only repeats Tassi's description.

SUMMARY

1. Best growth and production of perithecia of *Caryospora putaminum* occurred when cultures were grown at 25–28° C. on malt agars adjusted within the pH range of 5.0–6.0.

2. Growth on pea-extract agar differed in color from that on

malt-extract agar. Spermagonia and spermatia occur readily on the former with perithecia occurring only occasionally; on malt agar perithecia occur readily while spermagonia and spermatia are only of occasional occurrence.

3. Spermatia do not appear to be absolutely necessary for sexual reproduction and apparently are not concerned in dissemination.

4. Asci arise with or without crozier formation.

5. The most common number of spores per ascus is three, but from one to seven may occur.

6. A young three-spored ascus was observed to contain one nucleus per spore with five nuclei remaining in the epiplasm. Apparently the nucleus included in each spore divides and a median septum forms which gives rise to a two-celled ascospore, each cell of which contains one nucleus.

7. Most mycelial cells are uninucleate, but some appear to be multinucleate.

8. Division figures in paraphyses indicate a chromosome number of three or four.

9. *Caryospora minima* is proposed as a new species. It differs from other species of this genus mainly in spore size and shape.

10. Asci of *Caryospora minima* arise mainly by crozier formation and contain eight ascospores when mature. Each spore has two main cells and contains one nucleus per cell.

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PHOTOGRAPHS AND DESCRIPTIONS OF CUP-FUNGI—XXXV. A NEW SPECIES OF PATELLA

FRED J. SEAVER

(WITH 1 FIGURE)

For several years past the writer has been collecting, on burned areas in The New York Botanical Garden, a small brown *Patella* which superficially resembles *Sphaerospora brunnea,* but can at once be distinguished from this species by its ellipsoid spores. It was at first thought to be a dark colored form of *Patella melaloma,* but again is strikingly different in color and also has stubby, bristly hairs instead of the adpressed, blunt hairs which are so characteristic of *P. melaloma.*

After puzzling over this species for several seasons, the writer has finally decided to describe it since nothing has been encountered to which it could be referred.

Patella contradicta sp. nov.

Apothecia sessile, thickly gregarious or usually densely crowded, early becoming subdiscoid with the margin scarcely elevated, brown, reaching a diameter of 1–2 mm., rarely slightly larger, clothed about the margin with an inconspicuous fringe of hairs; hymenium plane or slightly concave, same color as the outside of the apothecium; hairs relatively short, bristly, tapering into a rather sharp tip, usually consisting of several cells, the basal one strongly swollen and subglobose, hyaline or subhyaline, usually not exceeding 50–80 μ in length and often much shorter; asci cylindric or subcylindric, reaching a length of 125–160 μ and a diameter of 12–16 μ, 8-spored; spores usually 1-seriate, broad ellipsoid, usually containing 2 oil-drops, about 10 $\times$12–16 μ; paraphyses enlarged above the tips reaching a diameter of 5 μ.

Apotheciis, sessilis, dense gregariis, orbicularis, brunneis, 1–2 mm. diam. margine pilis erectis, brevibus, hyalinis, erectis obsita; hymenio subplano

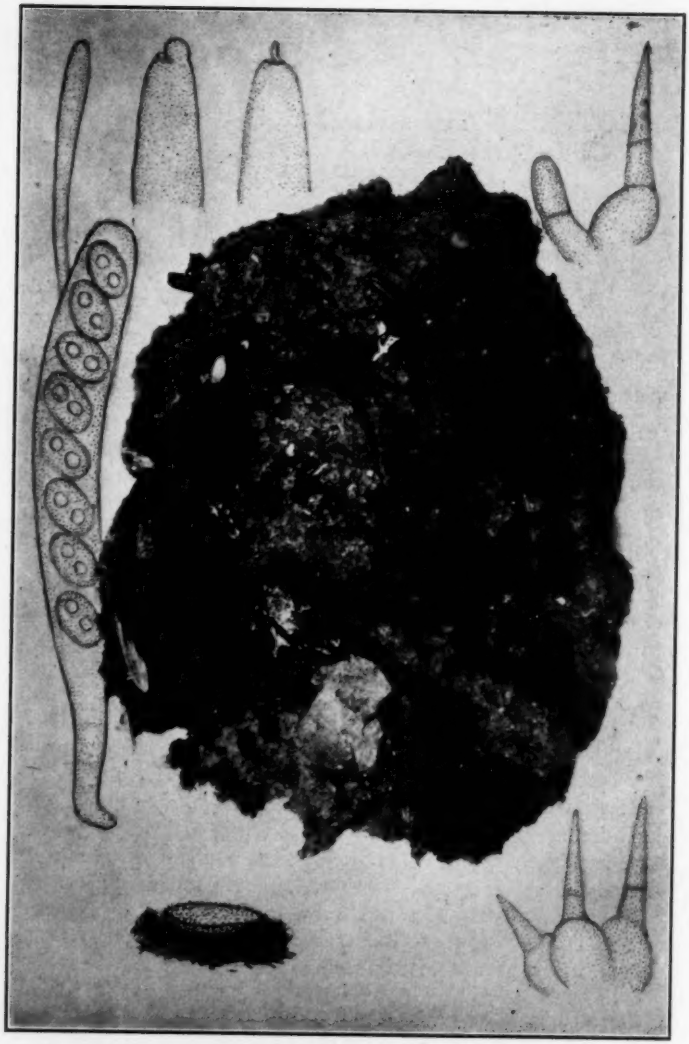

FIG. 1. *Patella contradicta.*

brunneo; asciis cylindraceis vel subcylindraceis, 8-sporiis, 12–16 × 125–160 μ; sporiis ellipticis 10 × 12–16 μ; paraphysibus clavulatis, 5 μ diam.

On soil where wood has been burned.

TYPE LOCALITY: The New York Botanical Garden.

DISTRIBUTION: Known only from the type locality.

NEW YORK BOTANICAL GARDEN

NOTES AND BRIEF ARTICLES

North American Flora

The final part of Volume 7 of North American Flora has recently appeared. Volume 7 consists of a monograph of the rusts and smuts of North America. The final part consists of a Bibliography by John Hendley Barnhart, and General Index by Gussie Mildred Miller. Mycologists have long complained that this volume, comprising more than a thousand pages, was not readily usable without the index, and the appearance of this part will doubtless be much appreciated by them. Full details may be had on request.—Fred J. Seaver.

Mounting Fluids and Double Cover-Glass Mounts

In a recent account presenting in detail a most commendable standardization of my method of mounting microscopic preparations between two cover-glasses Dr. Chupp[1] has proposed the name 'Shear's mounting fluid' for a formula associated by some persons with the name of Dr. C. L. Shear. This gives a certain nomenclatorial status to the formula (2 per cent potassium acetate in water 300 cc.; glycerine 120 cc.; 95 per cent ethyl alcohol 180 cc.). There is an equally useful formula of which the above appears to be an adaptation, and in view of my personal experience with the technique referred to, as well as familiarity with the media previously utilized, I feel obligated to present some historical details as pertinent.

'Shear's mounting fluid' described by Chupp actually differs slightly from that with which I had been familiar, and which has been used for years by many persons in Washington as a medium for semi-permanent mounts. This latter formula, presumably much older, is as follows: potassium acetate 10 grms.; water 500

[1] Chupp, C. Further note on double cover-glass mounts. Mycologia **32**: 269–270. 1940.

cc.; glycerine 200 cc.; 95 per cent ethyl alcohol 300 cc. This medium with just enough copper sulfate added to give it a light-blue tint made possible very satisfactory temporary preparations, but ringed mounts of from five to ten years old were observed in 1920 to be filled with blackish flecks that rendered them useless; the flecks were apparently of precipitated metallic copper. After 1921 the medium did not include the copper. It had also been customary to add to this medium small amounts of certain compatible stains (erythrosin, eosin, light-green, etc.) which had proved a suitable means of staining and preserving fungous structures at one operation. When in 1925 Colley [2] published this older formula and standardized the amount of erythrosin used he added as a footnote: "The medium has been used for years in the Bureau of Plant Industry, by Dr. C. L. Shear and others. The only modification of their formula is the addition of the erythrosin." I actually supplied Dr. Colley with the formula from the label of the stock bottle in the mycological laboratory. He had searched for a previous record of it, but without avail. Likewise several years earlier I had developed a lively curiosity with respect to the origin of this formula and its proper name. Search in the usual literature was fruitless and personal inquiries of my associates resulted in no definite answer. In 1921 I was told by Mrs. Flora W. Patterson, in charge of the Mycological Collections from 1896 to 1923, that she thought she had obtained it from Dr. C. L. Shear or from Mr. A. B. Seymour. When Dr. Shear was asked he replied that he had obtained it from Mrs. Patterson! I was told at a much later date by Mr. Seymour that he had never heard of this formula.

What name or whose name ought to be applied to this older formula still in use in Washington laboratories? The name 'Shear's mounting fluid' is preëmpted by the later modification and can not apply to it. It is possible that some obscure record of it may later be found but until that time it is only fitting to call it 'Patterson's mounting medium.'—WILLIAM W. DIEHL.

[2] Colley, R. H. A biometric comparison of the urediniospores of *Cronartium ribicola* and *Cronartium occidentale*. Jour. Agr. Res. **30**: 283–291. 1925.

NOTES ON GYMNOSPORANGIUM IN OKLAHOMA

In the spring of 1939 the author, with the aid of others, gathered together numerous collections of species of *Gymnosporangium* affecting various members of the genus *Juniperus* in Oklahoma. Several of these collections proved interesting in that new hosts and new localities for described species of the rust on Juniper were discovered. Because of these facts a list of some of the collections is hereby presented. Specimens of nearly all collections are deposited in the Mycological Herbarium, United States Department of Agriculture, Washington, D. C.

1. *Juniperus virginiana* L.

a. *Gymnosporangium clavipes* C. & P.—Collected April 6, 1939 and May 8, 1939, in Stillwater. Arthur [1] does not list this fungus in Oklahoma.

b. *Gymnosporangium globosum* Farl.—Collected March 26, 1939, at Stigler. This species is not so common as *G. Juniperi-virginianae.*

c. *Gymnosporangium Juniperi-virginianae* Schw.—Collected March 26, April 5, and May 8, 1939, in Stillwater. Evidently the commonest cedar rust in the state, especially since the host is native and commonly used for ornamental purposes and windbreaks.

d. *Gymnosporangium Nidus-avis* Thaxt.—Collected at the College Nursery on a dozen trees, May 8, 1939. These collections proved to be a problem, for the spores were borne in flat, irregularly-shaped horns arising from small, gall-like excrescenses along the branches. Such a habit is not usual. The spores were unusual and consisted of 2–4 cells. The several-celled spores had two pores near the septa of each cell except the uppermost cell which had the pore arranged apically. Specimens were sent to Dr. Kern for identification. Although no positive determination was made, he suggested that the rust was *G. Nidus-avis,* pointing out that this species was variable in habit and in the number of cells in each spore. Further study may reveal this fungus as new. Oklahoma is not listed as a locality for this rust by Arthur.[1]

[1] Arthur, J. C. Manual of the rusts in United States and Canada. p. 1–438. 1934.

2. *Juniperus virginiana* var. *Canaertii* Sénécl.

a. *Gymnosporangium Juniperi-virginianae* Schw.—Collected March 26, 1939, at Stigler.

3. *Juniperus virginiana* var. *glauca* Carr.

a. *Gymnosporangium Juniperi-virginianae* Schw. — Collected March 26, 1939, at Stigler.

4. *Juniperus virginiana* var. *globosa* Beiss.

a. *Gymnosporangium Juniperi-virginianae* Schw. — Collected March 26, 1939, at Stigler.

b. *Gymnosporangium floriforme* Thaxter.—Collected April 6, 1939, at Stillwater.

5. *Juniperus virginiana* var. *pyramidalis* Carr.

a. *Gymnosporangium Juniperi-virginianae* Schw.—Collected March 26, 1939, at Stigler.

6. *Juniperus chinensis* var. *Pfitzeriana* Spaeth.

a. *Gymnosporangium Juniperi-virginianae* Schw.—Collected March 26, 1939, at Stigler. This rust is not recorded on this host by Arthur.[1]

7. *Juniperus scopulorum* Sarg.

a. *Gymnosporangium Betheli* Kern.—Collected March 26, 1939, at Stigler. This fungus represents the first collection of *G. Betheli* in the state.

8. *Juniperus mexicana* Spreng.

a. *Gymnosporangium exiguum* Kern.—Collected April 7, 1939, in the Arbuckle Mountains. The host in this state is confined strictly to the region of the Arbuckles. Nearly every tree of the several hundred examined was infected in some degree. No tree, however, appeared to suffer any ill-effects from the presence of the rust. The identification of the rust was confirmed by Dr.

Kern. This report of the fungus in Oklahoma is the first. This host remains immune to *G. Juniperi-virginianae,* even though many trees of *Juniperus virginiana* affected by the rust are common in the same area with it. Because of this immunity to the destructive cedar-apple rust, nurserymen in the state are recommending the use of Mexican Juniper in place of the red cedar.—W. WINFIELD RAY.

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